

Th. Sect.

Thesis submitted for Degree of Ph.D.

The Degree of Ph.D. conferred -

22nd July, 1925.

A COMPARATIVE STUDY OF THE STEM STRUCTURE
OF THE GENUS CLEMATIS,

With special reference to the anatomical changes
induced by Vegetative Propagation.

by

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Thesis Presented for the Degree of Doctor of Philosophy
of the University of Edinburgh, 1925.



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INTRODUCTION.

The investigation of which the results are embodied in this paper was undertaken in an attempt to gain an insight into the problems of the propagation of Clematis by means of stem cuttings.

In order to be able to interpret the phenomena of regeneration induced by isolating portions of a plant, it is essential to investigate the normal anatomy in some detail. In any severed portion of a plant the first signs of abnormal cell activity in the tissues depart only slightly from the normal, and unless they can be detected and identified at their initiation it is impossible to define exactly the time and place of origin of the new growth.

Accordingly the first part of the paper will deal with the normal stem-anatomy of the genus. The second part, with the changes in the anatomy resulting from vegetative propagation, and the third part with some experiments in propagation.

MATERIAL. The material used was supplied almost entirely by the Royal Botanic Garden, Edinburgh. A few species were sent from Kew. The fresh material was supplemented by herbarium specimens where necessary.

Facilities for propagating were given by Mr. L.B. Stewart, head of the Propagation Department, Royal Botanic Garden, to whom the writer wishes to express her thanks.

METHODS. Wherever possible microchemical tests were used to supplement the ordinary stains. The Phloroglucin - HCl combination (with or without Iodine), the 2-solution Chlor-zinc-iodine, and Sudan III were used as a matter of routine. Many of the illustrations were made from temporary mounts of this character. It is believed that, in dealing with an essentially dynamic process, such as regeneration, that these microchemical reagents often give more significant information than the conventional permanent mount. Details of treatment are given for each figure. (For a list of reagents, stains etc., see page 52).

ILLUSTRATIONS. The photographs are on Eastman Commercial Film, either Orthochromatic or Panchromatic as required by the staining of the section, used in combination with the Wratten "M" Light Filters. Prints are on Vigorous Glossy Velox. Both negatives and prints developed with Tabloid " Rytol " Developer.

With three exceptions (figs. 39, 42, 43) the illustrations are the work of the writer. The negative of fig. 42 was made by Dr. R.J.D.Graham: the prints of figs. 39 and 43 were enlarged by Messrs. Lizars from the writer's negatives.

The writer is also indebted to Miss Stott for cutting some of the sections from which figs. 1-7 were made.

The writer wishes to record her thanks to Professor Wright Smith and his staff firstly for placing the resources of the Department at her disposal, and secondly for their consistently helpful advice and criticisms throughout the course of this investigation.

A. NORMAL ANATOMY.

I. GENERAL ANATOMY.

The genus *Clematis* includes about 170 species of cosmopolitan distribution. About 80 species are native of East Asia, and 20 of North America. The systematy of the genus has been dealt with by various authors, including Kuntze (11), Finet and Gagnepain (8), and Gray and Robinson (9). Horticultural papers include L.H. Bailey, (2), Jules le Bêlé (4), Lavallée (12), and Moore and Jackman (13). References to individual species occur so frequently in gardening literature that it is unnecessary to give separate citations.

The genus consists mainly of woody undershrubs, more or less climbing, with a certain number of woody herbs. The leaves are opposite and decussate, usually pinnately compound, lobed, or in some species entire; the majority with slender petioles, but a few sessile.

There is considerable difference in the shape, size and covering of the stem, corresponding to differences in habit etc., but the cross-section is fundamentally hexagonal, the angles being occupied by the principal bundles. The general stem-anatomy of the genus is remarkably consistent. Figures 1-7 have been made to illustrate the commonest types and some of the extremes. They all show transverse sections of stems in their first year of growth.

Figure 1. *C. coccinea* x *Scotfil* is a slender climber. The stem is six-sided, with little indentation between the angles. The stem has only six vascular bundles. The pith is lignif-

ied and shows starch. The pericyclic fibres are well marked and the first periderm is established.

C.patens is also a slender climber. The bundles are more widely spaced, and of the shield shape characteristic of the genus.

Figure 2. C.integrifolia x villida shows the six-sided stem very clearly, with six bundles, each with its cap of sclerenchyma. The pith and the medullary rays are lignified, and the first periderm is visible.

C.erlostema has less sclerenchyma, but the medullary rays are intensely lignified.

Figure 3. C.marata shows a more rounded stem, but still referable to a hexagonal foundation, with six small bundles connected by a dense band of lignified cells in the medulla. The cortex shows a suggestion of palisade-arrangement in the cells constituting the chlorenchyma, and the pericyclic fibres remain disconnected. The pith is heterogeneous, and remains so even in an old stem.

The structure of C.viticella is characteristic of a great many species. The definite hexagonal outline, with almost straight sides, the six large bundles of equal size, with large vessels and accompanying crescents of sclerenchyma: the solid homogeneous lignified pith, and the medullary rays less wide than the bundles, all go to make up a distinct

pattern. (Similar species are:- patens, coccinea, columbiana, hakonensis, virginiana etc.)

Figure 4. C. virginiana is similar to C.viticella, but it has twelve bundles instead of six.

C.Vitalba might be taken as the modal type of the genus, since this type of stem occurs most frequently in the species examined. It shows a six-pointed outline, with rounded ridges alternating with distinct furrows. The ridges are occupied by the six large bundles, and opposite the furrows are the six smaller bundles. The pericyclic fibres form a continuous band. The medullary rays are less in width than the bundles they separate, and the pith is often slightly hollow. This hollow centre is due to the tearing by rapid growth. The cells fringing the cavity are cellulosic, but the rest of the pith is lignified.

(Examples of "Vitalboid"stem:-
trullifera, lasiandra, Spooneri, Purdomi, Fremonti,
yunnanensis, Wattii, paniculata, pauciflora, montana,
gouriana, dioica, addisonii, Catesbayana etc.)

Figure 5. C.ranunculoides is a stout woody herb. The stem shows an extreme development of the "stellate" Vitalboid contour. Apart from the prolongation of the arms of the star, and the corresponding development of sclerenchyma, the stem does not differ from the general type of C.Vitalba.

C.afoliata, the most highly xerophytic member of the genus, is an interesting variant. The plant is of the "switch"

type, with minute deciduous leaves. The stem is approximately cylindrical, with slight ridges marking the twelve bundles. There is a thick cuticle and sunken stomata. The cortical cells approach the palisade form, and are divided off by flanges of sclerenchyma which extend right to the periphery. The bundles are somewhat narrower than in the preceding type, and the medulla is solid and homogeneously lignified. There is a development of phloem fibres forming a wavy band, one cell deep, all round the stele.

Figure 6. C.orientalis and C.napaulensis are climbers. They show twelve bundles (plus twelve secondary bundles in C.orientalis) in a more rounded stem. The hexagonal character is not entirely lost, but the ridges corresponding to the bundles are much less strongly marked than in C.Vitalba. These stems are approaching the cylindrical, slightly indented stems characteristic of the forms with many bundles. The pith in both cases is solid and homogeneously lignified, and the medullary rays much narrower than the bundles.

Figure 7. C.smilacifolia is a type with many bundles, large pith. The bundles are arranged more or less in a circle. C.heraclaeifolia also has many bundles, but here they are clearly arranged in hexagonal fashion. The pith in this species is very large and remains mostly cellulosic.

In addition to these types figured, there are a few species (recta, Flammula, ochroleuca, with definitely hollow

stems, which are cylindrical in outline.

Figure 7A is a synopsis of the main shapes of stem in the genus, showing the relation to the simple hexagonal outline.

VASCULAR PATTERN.

It may be seen by comparing the above figures, that the genus *Clematis* is characterized by the possession of a relatively simple vascular pattern, based upon a small number of primary bundles. (For the purposes of this description, the expression "primary bundle" is intended to refer strictly to those bundles which are first differentiated from the procambial strands and show true protoxylem vessels). As a result of the activity of the interfascicular cambium secondary bundles may be developed between the others and in more or less close connection with them. They link up with the primary bundles at the node.

Taking the vascular pattern as a criterion, the species of *Clematis* may be placed in two groups:-

A, with few bundles. B, with many bundles.

A.1). No additional bundles are formed by the interfascicular cambium, or

ii). A few secondary bundles arise, but the total is never more than double the original number. The most common type of increase in this group is a complete reduplication of the vascular bundles, a secondary bundle arising in each one of the medullary rays. These secondary bundles are always

much smaller than the primary, and are usually only developed when the stem is growing strongly.

B. The number of bundles constantly increases up to the end of the first year's growth, resulting in a ring of many bundles, with little difference in size. (The largest number observed being 42, in C.Flammula.)

Thus, in all the species examined, without exception, the number of bundles is either 6 or 12, or $6+x$, or $12+x$, x being a small number.

The constancy of the numbers is remarkable, especially in group A. In group B it is naturally less. If an axis is growing strongly, a bundle may be added, or if it is impoverished one may be dropped, but the outline of the pattern remains very strikingly.

The following table (Table I) gives particulars of the number of vascular bundles in the stem and the nature of the pith and rays in the types examined. Those forms which show an increasing number of bundles (i.e. group B, the many-bundled stems) are marked with an asterisk (*).

TABLE I.

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TABLE I.CLEMATIS: VASCULAR PATTERN.

The number in the second column is generally the result of several observations. When it is followed by a bracket, thus : 12(+x), it means that the the primary number is twelve and that x secondary bundles are present.

An asterisk (*) means a rising number of bundles; when the primary bundles are well-marked, it is written thus : 12*.

The numbers in the third column are the geographical regional numbers, according to the arrangement in the Herbarium of the Royal Botanic Garden, Edinburgh.

The contraction "homolig." means that the pith is uniform in structure and lignification.

Some varieties and hybrids have been listed with the species for convenience: garden names are in inverted commas.

Regional Classification.

- | | |
|-------------------------|---------------------------|
| 1. Europe. | 10. Tropical Africa. |
| 2. N.Africa and Orient. | 11. Mascarene Islands. |
| 3. N.Asia. | 12. South Africa. |
| 4. China(a), Japan(b). | 13. North America. |
| 5. India. | 14. Central America. |
| 6. Malaya. | 15. West Indies. |
| 7. Australia. | 16. E.Tropical S.America. |
| 8. New Zealand. | 17. W.Tropical S.America. |
| 9. Polynesia. | 18. Temperate S. America. |

TABLE.I.

I.		II.	III.	IV.
SPECIES.		Vasc. NO.	Reg. No.	PITH, MEDULLARY RAYS etc.
1.	acuminata	12		homolig.
2.	acutangula	12		
3.	addisonii	12		torn centre. Vitalboid
4.	aethusifolia	12		homolig.
5.	afoliata	12		homolig.
6.	alpina	12		homolig.
7.	angustifolia	12		
8.	apiifolia	12		homolig.
9.	aristata*	12*		
10.	Armandi*	12*		homolig.
11.	asplenifolia*	12*		homolig.
12.	balearica	12		homolig.
13.	Baldwini	6.		
14.	barbellata	6		hetero.
15.	Benthamiana*	24*		
16.	Bergeroni	6		homolig.
17.	Bigelovi	(6+6)		
18.	bonariensis	12+(8)		homolig.
19.	brachiata	12		
20.	brasiliiana	12		homolig.
21.	brevicaudata	12		

TABLE I. contd.

I	II	III	IV.
SPECIES	Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
22. <i>Buchananiana</i>	12		hollow cellulosic centre
23. <i>brevicaudata</i>	12(+4)		homolig.
24. <i>Cadmia</i>	6		cellulosic, rays very narrow. (cf. <i>lanuginosa</i>).
25. <i>calycina</i>	12		homolig., cells much thickened.
26. <i>campaniflora</i>	12		homolig.
27. <i>canaliculata</i>	12		homolig.
28. <i>Catesbayana</i>	12		homolig. Vitalboid.
29. <i>chinensis</i>	12		homolig.
30. <i>chrysocoma</i>	12		bundles widely spaced, rays much lignified.
31. <i>cirrrosa</i>	12(+4)		
32. <i>coccinea</i>	6,6(+6)		homolig.
33. <i>cochabambensis</i>	6(+6)		homolig.
34. <i>columbiana</i>	6		homolig., bundles widely spaced.
35. <i>connata*</i>	12*		hollow stem.
36. <i>velutina*</i>	12*		" "
37. <i>Graibiana</i>	12		homolig.
38. <i>Delavayi</i>	12(+8)		homolig.
39. <i>dioica*</i>	12-24*		hollow cellulosic centre.
40. <i>Drummondii</i>	12		homolig.
41. <i>eriosstema</i>	6		
42. <i>Fargesii</i>	12		homolig.
43. <i>fasciculiflora</i>	12		homolig.

TABLE I. contd.

I	II	III	IV
SPECIES	Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
44. Flammula*	12*	1	stem sometimes hollow.
45. flammulastrum *	24*	15	
46. foetida	12	8	homolig.
47. Forestii*	12*	5	homolig.
49. Fremonti	6	13	
50. fusca	12	4, 4a	homolig.
51. gentianoides	12	7	— herbaceous.
52. glauca	12	4	homolig.
53. glycerifolia*	12(+8)*	7	homolig.
54. glycerioides	12(+12)	9.	hetero.
55. gouriana	12	4, 5, 6	homolig. Vitalboid.
56. gracifolia	12		homolig.
57. grahami	12	16	homolig.
58. grata	12	5	homolig.
59. graveolens	12	5	homolig.
60. grewiaeflora	12	5	homolig.
61. grossa	12(+12)	14	homolig.
62. hakonensis (lanuginosa x viticella)	6	—	homolig. viticelloid.
63. hedysarifolia*	12*	5	hollow cellulosic centre.
64. Henryi	6	4	homolig.
65. heraclaeifolia*	12*	4, 4a	hetero., mainly cellulosic.

TABLE I. contd.

I		II	III	IV
SPECIES.		Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
66.	Hillarii	12	16	homolig
67.	indivisa lobata	12(8)	8	
68.	integrifolia	12	1,3	
69.	(integrifolia x villida)	6	—	
70.	((integrifolia x viticella) x florida)	12	—	
71.	intermedia	12	?	homolig.
72.	ispahanica	12	2	homolig.
73.	japonica	6	4a	homolig. rays very wide. (cf. chrysocoma)
74.	Kerriana	12	4,5	homolig.
75.	Kirkii*	12*	10	hetero., mainly cellulosic.
76.	lanuginosa	6	4	hetero. cellulosic, rays very narrow. (cf. Cadmia).
77.	lasiandra	12	4a	homolig.
78.	lasiantha	12	13	homolig.
79.	leiocarpa	12	4	hetero.?
80.	ligusticifolia	12	13	homolig. Vitalboid.
81.	mandschurica *	12*	4	hollow stem.
82.	marata	6	8	hetero. lig. only between bundles
83.	mauritiana	12	11	homolig.
84.	meyeniana	12	4a	homolig.

TABLE I.contd.

	I SPECIES.	II	III	IV
		Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
85.	montana	12	5	homolig. Vitalboid.
86.	rubens	12	5	" "
87.	Wilsoni	12	5	" "
88.	"Mme. Baron Veillard"	6	—	
89.	"Mrs. George Jackman"	12	—	
90.	"Mrs. Villiers	6	—	
91.	nannophylla	6(+6)	4	homolig., dense medullary rays.
92.	napaulensis	12	5	homolig.
93.	nutans thyrsoides	12	5	homolig.
94.	ochroleuca*	12*	13	hollow stem.
95.	orientalis	12	5	homolig.
96.	paniculata	12	4, 4a.	hollow cellulosic centre. Vitalboid
97.	patens	6	4a	homolig.
98.	parviflora	12	8	homolig.
99.	parviloba	12	4	homolig.
100.	pauciflora	12	13	homolig.
101.	pavoliniana	12	4	homolig.
102.	Pitcheri	12	13	homolig.
103.	pogonandra	6	4	hollow cellulosic centre.
104.	pseudopogonandra	6	4	hollow cellulosic centre.

TABLE I. contd.

I		II	III	IV
SPECIES.		Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
105.	pterantha	12(+12)	4	hollow cellulosic centre.
106.	Purdomi	12	4	homolig.
107.	puberula	12	5	homolig.
108.	quinquefoliata	12	4	homolig.
109.	ranunculoides	12		homolig.
110.	recta*	12*	4	hollow stem.
111.	flore plena*	12*	4	" "
112.	mandschurica*	12*	4	" "
113.	reticulata	12	13	homolig.
114.	rubifolia	12	4	homolig.
115.	saxicola	12(+8)	11	
116.	Scottii	6	13	
117.	Scottii x coccinea	6	—	
118.	sericea	12	14	homolig.
119.	serratifolia	12	4	homolig.
120.	simensis	12(+12)	10	homolig.(cf. napaulensis)
121.	smilacifolia *	12*	4,5,6	hetero.
122.	songarica	12	3	homolig.
123.	Stanleyi	12(+12)	12.	homolig.
124.	tangutica	12	4	homolig.
125.	ternata	12	4a	homolig.

TABLE I. contd.

I	II	III	IV
SPECIES	Vasc. No.	Reg. No.	PITH, MEDULLARY RAYS etc.
126. Thunbergi	12(+8)	10	homolig.
127. trullifera	12	4	hollow cellulosic centre. (Vitalboid).
128. uncinata	12	4	homolig.
129. urophylla	12(+12)	4	homolig.
130. vedrariensis	12		homolig.
131. Viorna	12	13	hollow cellulosic centre.
132. Vitalba	12	1	hollow cellulosic centre.
133. viticella	6	1,2,3	homolig.
134. virginiana	12	13,16.	homolig.
135. Wattii.	12(+12)	6	Vitalboid.
136. Wilfordi	12	?	homolig.
137. Wightiana	12	10	homolig.
138. yunnanensis	12	4,5.	hollow cellulosic centre, (Vitalboid).
			<p data-bbox="779 1560 1179 1600"><u>ANALYSIS OF NUMBERS.</u></p> <p data-bbox="749 1659 1253 1799"> 6 bundles in stem.....25. 12 " " "94. many " " "19. <u>138.</u> </p>

It will be seen from the above table that the greater number (94 out of 138) of species examined possessed a vascular pattern based on twelve bundles, and this is accordingly taken as the central type for the genus. This pattern may be expressed diagrammatically for reference thus:-

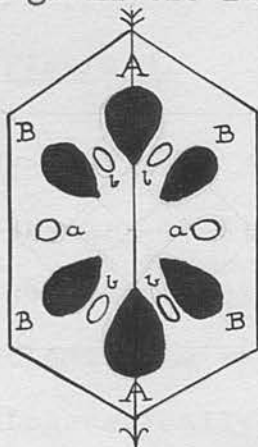


Figure 8.

A,A, represent the median bundles of the nearest leaf-trace: B,B, the lateral trace-bundles: a, and b, the corresponding bundles from the next node above. The hexagonal stem shows two axes of symmetry, the longer passing through the median leaf-trace bundles, and the shorter at right angles to this. From the decussate phyllotaxis it follows that the long axes of symmetry of two successive internodes are at right angles to one another.

This 12-bundled stem represents the pattern of C.Vit-alba and similar forms. Expressed in symbols this becomes:-

$$2A + 2a + 4B + 4b = 12.$$

The six-bundled stem may be formulated as

$$2A + 4B = 6.$$

The diagram of the six-bundle stem is :-

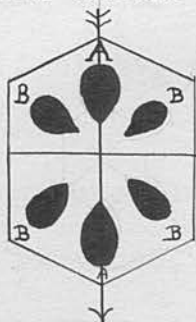


Figure 9.

Even in the many-bundled stems it is usually possible to distinguish the primary bundles and to assign the secondary bundles to their appropriate grouping.

In order to indicate the way in which the many -bundled stem is derived (ontogenetically) from the twelve-bundled, Figure 10 shows a series of sections from the stem of C. Armandi. By sectioning sufficiently near the apex, the stage with twelve primary bundles is obtained. Progressively older internodes show twenty and twenty-eight bundles respectively. The primary bundles are labelled A and B as described above, the secondary bundles x and y. A stem such as this can be formulated:-

$$\text{Fig. 10, 2). } 2A + 2(a+2) + 4(B+1) + 4b = 20.$$

$$3). 2(A+2) + 2(a+2) + 4(B+2) + 4b = 28.$$

The brackets indicate the approximate groupings of the secondary bundles with their related primary bundles.

It is not possible to indicate any general rule for the sequence of origin of secondary bundles.

So far as can be judged on the material examined, the type of pattern(few or many-bundled) is constant for a given

species. The vascular number appears to be reasonably constant in the adult plant, within the limits of variation indicated above.

Some interesting juvenile transitions were noted in the youngest plants examined, which were in a batch of two-year old plants (from seed collected by Mr. George Forrest) at the Royal Botanic Garden. In specimens of Clematis grata, C. montana, C. trullifera and C. yunnanensis there were stems which showed only one leaf at the nodes at the base of the branch. In one plant of C. yunnanensis, the first-formed node above the cotyledons had one leaf only and no sign of a second. The node above this had one leaf (at an angle of 90° with the first) and a ridge opposite to it. The third node had again one developed leaf and a ridge. Only at the apex and on the branchlets in the axils of the leaves did the opposite and decussate character appear. The internal structure of this young plant was interesting. At the base the stem was triangular instead of hexagonal. There were three prominent bundles instead of six, the remaining three being small. Adult plants of this same species had the normal decussate phyllotaxis and the stem showed twelve vascular bundles. It is evident from this and other cases that the vascular number is a well-established characteristic.

In all the other seedling plants examined, the decussate phyllotaxis was present from the beginning. It is hoped in a later paper to give an account of the seedling anatomy, which

promises to be interesting, as showing the transition from a diarch xylem plate in the root to the six or twelve bundles in the stem. (See Fig.12 for the diarch root).

NODAL ANATOMY.

All the bundles of the stem of Clematis are related to the leaf-traces: there are no purely cauline bundles.

Taking the six-bundle type first as being the simplest, the course of the bundles through the stem is as follows. Six bundles enter the stem at the node. They run through one internode only, and at the next node below they divide into two. The entering leaf-trace strands receive one half-bundle on each side, so that the ring of six bundles is re-formed directly. (For a more detailed description, with plan and sections, see Fig. 13 and 14. The node of C.viticella is figured by Nageli (14)).

In the case of C.Vitalba and others with twelve bundles, the six leaf-trace strands run through two internodes before losing their identity. Thus two sets of six bundles are present in each internode, commonly distinguishable by their difference in size (. Figs. 15,16. cf. also de Bary (3)).

In the stem with many bundles, the node is naturally more complicated. The principal leaf-trace bundles behave as in the twelve-bundle stem, and the secondary bundles, as mentioned above, all take part in the fusion and redistribution. A stem of C.smilacifolia has been figured and described in detail in Figs. 17,18.

The twelve-bundled type of stem has been taken as the modal type of the genus simply on the ground of frequency of occurrence. It is not intended to imply that those species which have only six bundles are relatively primitive, or those which have $12+x$ bundles more advanced than the prevailing type. It is easy to derive the many-bundled stem from the twelve-bundle type, but whether the six-bundle type is a reduction from the twelve or the developmental starting-point of the whole series is another matter, and one which the writer cannot decide on the evidence from the adult plant alone.

A. NORMAL ANATOMY. 2.HISTOLOGY.

EPIDERMIS. A single layer only, with thin cuticle (except in C.afoliata), and few stomata. (Fig.19,e). The stomata do not seem to be related in any constant way to the ridges and furrows of the stem, nor do they show any special xerophytic adaptation, except in C.afoliata. In this species they are sunken, and the cuticle is thrown into ridges on the epidermal cells. (Fig.20). Some species, as C.lanuginosa, C.grewiaeflora, are covered with downy hairs, which are simply prolongations of epidermal cells.

CORTEX. i). Collenchyma. This is always present in the stem as one or two layers of cells immediately under the epidermis, widening out into flanges opposite the main bundles. The cells are typically thickened at the corners and give an intense cellulose reaction with iodine and sulphuric acid or with chlor-zinc-iodine. (Fig. 19,col.)

ii). Parenchyma. The primary cortex in the entire genus contains chlorophyll and is assimilatory. The cells are thin-walled, in most species nearly isodiametric, but in C.marata (Fig.3), and C.afoliata (Fig.5), there is an almost palisade-like arrangement of the chlorenchyma. The latter species is very xerophytic, with a whip-lash stem and minute deciduous leaves. The photosynthetic tissue is cut up into blocks by the flanges of schlerenchyma which subtend the vascular bundles. Diels (4) figures this species,

but the illustration is only diagrammatic.

iii). Starch-sheath. The starch-sheath begins to differentiate in the apical meristem about the level of the first bud-primordium. (Fig.21). At the immediate apex the starch is distributed uniformly throughout the tissues. Once the starch-sheath appears, the distribution of starch in the young stem is influenced by it. In the young and rapidly extending internodes little starch is deposited in the tissues, and what does occur appears in the starch-sheath and in the tissues external to it. At the node the starch-sheath breaks down, with the entrance of the leaf-traces. The first break appears opposite the bud, and the gap widens until the sheath is only visible as an arc subtending the a-bundles. (See Fig.8). With the breaking down of the sheath, the starch is no longer confined to the cortex, but travels through the medullary rays into the pith. As the bundle-ring re-forms below the node, so does the starch-sheath link up, and when it is once more complete the bulk of the starch is again in the cortex. After rapid growth has ceased in the inter-node, starch gradually accumulates in all the tissues, intrastelar as well as extra-stelar, and it is no longer possible to distinguish the starch-sheath as a morphological boundary. Figure 22 shows a partly differentiated bundle of C. heracleifolia, with a very marked starch-sheath.

The question arises as to how far this starch-sheath can be considered as equivalent to an endodermis.

Considered as a barrier to the passage of starch, (or rather of those soluble carbohydrates from which starch may be formed), it is evidently functional only when the excess of these carbohydrates in the stem is not great. The fact that, once active growth in length has ceased, starch accumulates in great quantity in the pith shows that the starch-sheath is only efficient within narrow limits. There is no sign of a Casparian strip in any normal stem. In an attempt to induce its development by etiolation, a strongly growing tip of C. Forrestii was enclosed in a black paper bag and allowed to grow in the dark for four weeks. Even after this treatment no Casparian strip was discernible. It may therefore be said that the starch-sheath is neither structurally nor functionally the equivalent of an endodermis.

STELE. 1). Pericycle. In all the species examined there is a marked development of pericyclic fibres. These fibres give a lignin reaction with Phloroglucin-HCl and with Chlorzinc-iodine. The walls are typically thick (8-10 μ), with slit pits. The lumen of the cell is small. At the same time, starch is sometimes found in the cells. Moreover, the cell-wall diminishes in thickness and gives a less intense reaction (or none at all) with phloroglucin after etiolation. The significance of this will be discussed in the section on propagation.

In the young stem the pericyclic fibres first appear

as separate crescents opposite the bundles. As the stem matures, these crescents may become united into a continuous wavy band. The later developed portions of the band are on the whole less strongly thickened than the original groups of fibres.

A most characteristic and typical continuous pericyclic band is seen in Clematis Vitalba. (Fig.4). For examples of discontinuous pericyclic fibres, see C.marata, (Fig.3), C.afoliata (Fig.5), C.smilacifolia (Fig.19). The last three stems figured are still in their first year, but in these cases the condition remains the same until the development of periderm, which does not necessarily occur in the first year. C.napaulensis shows well the original dense arcs of fibres and the less strongly thickened connections.

Periderm arises from a cork cambium which develops in the inner layers of the pericycle. This pericyclic origin of the first phellogen is constant throughout the genus, except in C.smilacifolia. In this species the oldest available stem (which had a girth of 10cm.) was examined. There was no true "bark". A phellogen had arisen sub-epidermally, and produced two to three layers of cells between it and the original collenchyma. The epidermis was still present, but cracked in places. The collenchymatous cells were pulled out parallel to the surface of the stem. Where cracks occurred the cells of the cortex were partially infiltrated with brown substances, but gave no suberin test with Sudan III.

This position of the periderm is unique among the species of *Clematis* examined.

Usually the first season's growth is terminated by the development of this deep-seated cork layer, but sometimes its appearance is delayed. (For first periderm, see Figs. 1, 2, 3). In the majority of cases the whole of the tissues external to the pericycle and even the pericyclic fibres themselves are sloughed off, but occasionally the epidermis remains intact although two or more complete periderms are present.

In the second and each succeeding year the phellogen is developed in the phloem, just within the corresponding annual group of phloem fibres. (Fig. 23). Each phellogen contributes three to six layers of cork to the outside. There is no centripetal development of secondary cortex. The cork cambiums span both the wide primary rays and the secondary wood rays, and consequently the periderm tends to tear away in strips, corresponding in number to the rays. This furrowed, string-bark is characteristic of the genus, and is specially marked in the strongly climbing species such as Vitalba, montana, etc. In the bushy and herbaceous types the bark is smoother.

11). Vascular Bundle.

a). Phloem. The phloem consists mainly of sieve tubes and phloem parenchyma, with few definite companion cells. During the first year's growth fibres are differentiated in the phloem, originating as distinct patches on either side of

the bundle.(Fig.19,pf). By the end of the season there is usually a complete hoop surrounding the phloem. (see C.afoliata, Fig.5).

b).Cambium. The development of the interfascicular cambium proceeds from the edges of the fascicular cambium in the usual way. The part played by the interfascicular cambium in the development of the secondary bundles has been referred to above.

Clematis shows a stratified cambium, with two types of initials, fusiform and ray. The fusiform initials average $270\mu \times 23\mu$ (tangential diameter) $\times 15\mu$ (radial diameter). The ray initials are about $39 \times 34\mu$, that is, approximately isodiametric. The majority of the divisions are tangential, and the cambium ring increases in girth by radio-longitudinal divisions and expansion of the daughter cells, as described by Bailey (1) for dicotyledons which are highly specialized anatomically. This lateral meristem as seen in radial longitudinal section appears to consist of a band of five to six undifferentiated cells with large nuclei. On treating with Chlor-zinc-iodine a gradation of tint is seen in the cell-walls. One or at most two vertical tiers of cells in the middle of the band have particularly dense protoplasm, and their walls do not stain appreciably with this reagent. The cells which are situated centrally to this tier, which are in the process of becoming xylem elements, show first a distinct cellulose reaction before lignification sets in.

The cellulose walls may be appreciably thickened and show the beginnings of pitting. A cell has been drawn and photographed which shows lignified pitted wall, nucleus, protoplasm and included starch, while its neighbouring cell is still cellulosic. (Fig.24). Centrifugally this increase in the cellulosic reaction of the walls also occurs. The immature phloem shows the same stratified arrangement as the tissues from which it is derived, and the beginning of the sieve-plates are visible at this stage. (Fig.24,B). The plate is about $40-90\mu$ long, the perforations $0.3-0.5\mu$. Vacuolation is only beginning in the cytoplasm.

For the purposes of the section on propagation it is proposed to call the whole of this lateral meristem the "cambial layer", since the band of five or six cells which includes the "cambium" and its first products of division seem to react equally to the stimulus of wounding in the making of a cutting.

c). Xylem. The shape of the xylem mass of the primary bundle is similar throughout the genus. It is in the form of a triangular shield, the point being occupied by the protoxylem. The upper edge of the shield is concave, and the corners are frequently marked by two large vessels.

The number of protoxylem elements is small. The vessels are typically long and slender, the walls annularly or spirally thickened and frequently collapsed. (Fig.30).

The metaxylem consists of vessels, some of very wide

lumen and others narrower: tracheids, fibre-tracheids grading into fibres, and cells which retain their protoplasmic contents and which must accordingly be interpreted as xylem parenchyma.

The average diameter of the large vessels is 136μ , but some occur up to 200μ . The length of the segments varies from $300-500\mu$, average 400μ . The terminal walls, as indicated by the remaining septa, are set at an angle of $60-90^\circ$. The terminal perforations are large simple pores. The lateral communication with vessel and vessel or vessel and tracheid is by bordered pits: With xylem parenchyma, ray parenchyma or fibre, by simple pits. The pits are closely packed, and wavy lines of tertiary thickening appear between them, in addition to somewhat regular fine striations forming a mesh. (Figs. 24, 26, 27, 30).

The smaller vessels are of narrower diameter ($70-100\mu$), but the length of the segments and the relation and perforation of the terminal wall are the same as in the larger vessels. Sometimes the thickening is a simple reticulation without bordered pits.

The tracheids proper average $400 \times 22\mu$. They have tapering ends, at an angle of 30° or less. The terminal walls as well as the others show simple pits when they abut on a medullary ray cell, on a xylem parenchyma cell, or on a secondary wood ray. When they abut on a vessel the pits are bordered. Mixed with these tracheids are others which have a very narrow lumen, and these grade into true fibres.

The fibres average $554 \times 20\mu$, the empty cavity being about 6μ in diameter. The end walls are extremely pointed, and the slit pits almost indistinguishable.

The proportion of tracheids to vessels varies with the habit of the species. The extreme liane types show a greater proportion of large pitted vessels than the herbaceous species.

Even in the oldest stems the bundles maintain their identity; no continuous xylem ring being formed. The wide primary rays persist, and the xylem masses are also penetrated by the secondary wood rays. (See next section).

iii). Rays. The rays in the stem of *Clematis* are of two kinds - the wide primary rays extending right from the medulla to the periderm, and the secondary wood rays. Both types are multiseriate.

In the young stem the rays do not play such an important part as in the old woody cylinder, but the topographical relations of the bundles and the wide primary rays are determined in the young stem. Up to the end of the first year's growth each bundle is flanked by a mass of storage parenchyma which is in connection with the pith. There is a considerable diversity in the organization of the rays and pith throughout the genus, but the primary rays are always multiseriate. (For the condition in a young stem, see *C. smilacifolia*, Fig. 19).

The wide ray abuts directly on the medulla. The cells

composing it have their long axes placed radially, in direct contrast to the pith cells which are elongated vertically. (Figs. 26,27). The average dimensions of the ray cells are $60-70\mu$ long, 40μ wide, and 30μ deep. The cells are often pulled out into diamond shape, the end walls being at an angle of 45° . The row of cells immediately flanking the wood is usually only 20μ wide. Lignification proceeds centrifugally, lagging considerably behind the development of secondary wood. (Fig.26). The lignified walls are about 5μ thick. The middle lamella is very distinct, and there are numerous simple pits, on all faces of the cell. The non-lignified elements are of similar size and shape, but with thinner, cellulosic walls. Starch is densely stored in the lignified portion of the ray, but is scanty in the non-lignified part. (Fig.26).

The secondary wood ray is a product of cambial activity in the second and succeeding years of growth of the stem. As a result of the development of these small rays, the xylem-masses are partially cleft into smaller segments. The cells which compose the secondary rays are similar in size, shape, pitting and storage function to those described above.

The activity of the cambium is less where it crosses a ray, resulting in the formation of depressed segments in the cylinder. (Fig.23). In consequence of the continuity of the wide rays, even the oldest stems do not present a solid and uniform mass of wood, but are liable to cleavage in the lines of the rays. (See Sinnott and Bailey (16), and Solereder(18)).

iv). Pith. There is some diversity of constitution in the pith throughout the genus. The different types may be grouped as follows:-

I. The pith, which is at first uniformly cellulosic (this applies to all groups), becomes rapidly and uniformly lignified, giving a positive reaction with Phloroglucin-HCl and with Chlor-zinc-iodine. Out of 138 species examined, 78 answer to this description. With this type may be placed those species with lignified pith, in which a slight hollow is present in the centre, torn by extra rapid growth, and lined with cellulosic cells. This is the type of Clematis Vitalba, also C.dioica, montana, Spooneri, trullifera, yunnanensis etc. (It should be mentioned here that a small patch of cells surrounding each protoxylem point often remains cellulosic.

II. Lignified externally, but solid cellulosic centre.

a). few bundles, very widely spaced:- C.marata, chryso-coma, pauciflora, japonica etc.

b). many bundles:- heracleifolia, Kirkii etc.

III. Lignified externally, hollow centre (pipestem), cells round hollow not lignified:- C.recta, Flammula, ochroleuca.

IV. Heterogeneous, mainly cellulosic, only a cell here and there lignified:- lanuginosa, Cadmia.

V. Heterogeneous, a ring of cells at the periphery of the pith lagging behind the rest in lignification:- smilacifolia.

This last case is interesting from the point of view of propagation. The band of cells referred to give a brilliant cellulosic reaction, and show large simple pits, protoplasm and nuclei. In propagation these cells take part in callus formation. As the stem gets older these cells become lignified, and in propagating from such mature wood the pith does not form any callus.

The pith cells are cylindrical, and show a gradation in size from the centre outwards (Fig.25). The central cells average $236\mu \times 40\mu$, and the peripheral cells $236\mu \times 20\mu$. The end walls are at right angles to the long axis, and all walls show simple pits. Although these cells give a lignin reaction, they cannot be considered to be entirely removed from the current of metabolism, since cell contents can be seen in many, and much starch is stored in the pith. Unless this reserve is to be regarded as entirely lost to the plant, it must be conceded that even a cell which is both histologically and chemically highly differentiated is capable of storing and yielding up reserves when required.

B. ANATOMICAL EFFECTS OF VEGETATIVE PROPAGATION.

B. ANATOMICAL EFFECTS OF VEGETATIVE PROPAGATION.

The most common commercial method of propagating Clematis is by grafting on to the fleshy roots of C. Vitalba or C. integrifolia. This method is used for rapidly increasing the stock of a new or specially fine variety. Layering is also practiced. The long branches are pegged down and covered with soil. The stem is slightly twisted, to crack the inner bark, and promote rooting. Stem cuttings may also be used, either of half-ripe wood or the soft tips taken at the very beginning of the growing season. The latter require a temperature of 70° F. or so. They are rather liable to damp off, but on the other hand they strike quickly, and having the whole season before them, make better plants the first year than the older cuttings. Cuttings of the half-ripened wood are the most reliable. They may be struck in fibre or sand, at a soil (or compost) temperature of 60° F. or less according to the species. The best month in which to take cuttings varies with the species: no general rule can be given. By courtesy of Mr. L.B. Stewart, the following extract from the records of the Propagating Department, Royal Botanic Garden, is included.

Batches of six cuttings were put in each month throughout the season 1924. The most successful and the least successful months are noted for six species.

Month in which Cuttings are taken.

<u>Species.</u>	<u>Maximum rooting.</u>	<u>Minimum rooting.</u> (dead).
Armandi	1/6, Feb., Mar., July.	6/6 June.
campaniflora	_____	6/6 June.
grata	6/6, July.	6/6 June.
Jouniana	4/6, July.	6/6 May.
lasiandra	1/6, Feb.	6/6, June, July August.
montana	_____	6/6, May June.

It was stated by the writer in a preliminary paper(17) that, under ordinary circumstances, stem-cuttings of Clematis will not root at the node. This requires modification. It appears that many commercial houses still use nodal cuttings, in spite of the fact that such cuttings are very much slower in rooting than those made at the internode. (By a "nodal" cutting is meant one which is cut hard back to the leaf-bases. That, is, the node as defined by the gardener and not by the botanist). The usual course of events is that the end of the cutting rots away, and rooting actually takes place above the node. This is an unnecessarily slow method, since cuttings made one inch or so below the node can be rooted in 3-4 weeks and established in 6-8 weeks.

Numerous references to horticultural literature will be found in Miss Chandler's résumé of publication up to 1913. (5).

CALLUS FORMATION.

The amount of callus formed by stem cuttings varies with the age of the wood taken, the amount of food reserves (starch etc.) as well as with the anatomical structure of the species. Upon the latter depend the exact tissues which contribute to the callus. Other factors no doubt come into play, but these are fairly obvious and some comparison is possible.

The age of the wood taken for a cutting and the amount of food reserves are closely linked. The amount of starch in a stem increases with age, but it is of course very dependent upon the external and highly variable factors of light, temperature and moisture. Thus it often happens that, of two stems which have reached a similar stage of anatomical differentiation, one may have more reserve starch than the other, and may behave differently in propagation. There is an undoubted correlation between the amount of callus formed by any given cutting and the amount of starch present in the tissues, though it is not yet possible to give a quantitative relation.

The following list of species propagated by the writer gives some idea of the varying degrees of callus formation. The cuttings were of half-ripened wood. They were put in cocoa-nut fibre, in a propagating case with a bottom heat of about 65°F. The times on the second column are the number of days (average of 6-10 cuttings) required to cover the cut end with callus.

SPECIES.TIME and DEGREE OF CALLUS FORMATION.

1. <i>afoliata</i>	very slow, small amount formed.
2. <i>Armandi</i>	14 days.
3. <i>campaniflora alba</i>	died off.
4. <i>Delavayi</i>	18 days.
5. <i>grata</i>	8 days.
6. <i>grewiaeflora</i>	18 days.
7. <i>Hilarii</i>	14 days, formed large callus.
8. <i>lasiandra</i>	8 days.
9. <i>montana</i>	8 days.
10. <i>montana Wilsoni</i>	died.
11. <i>napaulensis</i>	8 days.
12. <i>nutans thyrsoidea</i>	died.
13. <i>orientalis</i>	8 days.
14. <i>Purdomi.</i>	16 days.
15. <i>ranunculoides</i>	10 days, formed large callus.
16. <i>smilacifolia</i>	19 days, small amount formed.
17. <i>Spooneri</i>	18 days.
18. <i>uncinata.</i>	very slow, small amount formed.

The above species were all propagated in October and November 1923.

Figure 31 shows early stages in callus and root formation in *C. smilacifolia*, *C. Spooneri*, *C. Hilarii*.

Anatomically speaking, the genus includes a wide range of forms, from the liane type with large distinct bundles, few in number, persistent primary rays and relatively small lignified pith; to types with many bundles, hollow pith; and a small but interesting section with widely spaced bundles, medullary rays much lignified, and a solid but heterogeneous pith. The first class is numerically by far the most important, and all the cases which have been observed to fall within it are very much alike in their anatomical responses to propagation. (e.g. C. montana, etc.) To the second class belong C. recta, Flammula, ochroleuca, which have not yet been propagated by the writer. The third group includes C. japonica, chrysocoma, Purdomi, etc. The third group differs from the first only in that the pith contributes to the callus. This classification is, of course, acceptable only in the most general sense, but in considering the effects of propagation it serves to bring together instances which at first sight seem to depart from the general scheme.

The reactions of an isolated portion of a plant to the stimulus of cutting can be grouped under three heads; chemical, histological and morphological. These three groups of changes show a time-sequence in their appearance. The first observable changes in a cutting, before any callus formation begins, are those indicating the death-changes in the divided protoplasts and the altered metabolism of the injured cells above the cut. Even the sharpest of knives causes a

certain amount of bruising besides the actual cutting of cells. In addition to this direct effect of injury, the access of atmospheric oxygen to the cut surface leads to the formation of a suberin seal, as described by Priestley for the potato.(15). The presence of this seal seems to be essential for meristematic activity. In *Clematis* the seal is established in three to four days, and the first signs of abnormal cell divisions have been observed (in *Clematis x milacifolia*) after eight days at 65° F. The origin and development of callus has been worked out in detail for this form, and the following description is taken from it.

1). A section of a cutting which had been kept at a temperature of 65° F. for eight days shows the beginning of activity in the interfascicular cambium. (Fig.33). Where the cut has passed through living cells, the protoplasm is seen to be disintegrated into brownish traces. The cells immediately above this have their protoplasm and nuclei contracted into a round mass. The third and fourth cells (in the same vertical series) show an oblique tangential wall, the two daughter nuclei being side by side about the centre of the new wall. This division departs only slightly from the normal tangential division of the cambium, and represents the earliest detectable stage in the initiation of callus. A transverse section confirms the interfascicular cambium as the starting-point. (Fig.32).

2). A similar cutting, sectioned after thirteen days, shows irregular transverse divisions in the cells from the interfascicular cambium, giving a mass of about six to eight cells which protruded beyond the cut. The ring of cellulose cells in the pith (see above, p.33.) show cell-divisions. The starch contents are much diminished in the dividing cells. In these pith cells, which have thick walls, the new walls appear first vertically in irregular planes, and are much thinner than the old, so that the cells appear to be cut up into halves or quadrants, much like a spore-mother-cell. By this time the vascular cambium has become active, the divisions spreading from the edges to the middle of the bundle.

3). The growth-thrust of the new tissue formation crushes the cells in its path, and arcs of crushed tissue approximate opposite the centre of the vascular bundle. (Fig.34).

4). The meristematic activity extends centrifugally in the medullary rays, and irregular divisions are seen in the phloem and cortex, so that all these tissues ultimately contribute something to the callus. The callus grows in lobed masses corresponding to the wide rays.

C. smilacifolia differs from the others of the species studied in forming very little callus. In a one-year old cutting of C.Smilacifolia, which had filled a four-inch pot with roots (Fig.37), a depth of only fifteen cells has been

formed, counting from the edge of the cut.

5). In other forms where a good bulk of callus is formed the outer layers become suberized and tracheids are developed in it. Figure 35 shows a longitudinal section of a three-months old callus of C.gracifolia, which indicates well the loose, unorganized growth and the corky nature of the outer layers. Many of the cells are packed with starch. The patches of meristem show up lighter than the rest, since they do not contain starch. Tracheids are seen in irregular groups, in no definite relation to the wood of the stem. The cambium of the stem, at the point where it passes over into callus, is giving rise to tracheids, but these do not apparently link up with the callus tracheids proper.

ORIGIN OF ADVENTITIOUS ROOTS.

The adventitious roots in *Clematis* originate from the fascicular cambium of the stem. More than twenty root-primordia have been sectioned in the earliest stages (that is, before they have broken through the epidermis), and in all cases the new mass of embryonic cells was in continuity with the cambium of a vascular bundle. (Fig. 36). The earliest detectable stage shows a few embryonic cells extending from the cambium layer into the medullary ray. These new cells are characterized by their size ($3-5\mu$), extremely dense protoplasm, and walls which do not give the direct cellulose reaction with chlor-zinc-iodine. There is no sign of distortion in the cambial arc, which would indicate that cell-division had begun outside the bundle and was spreading inwards. On the other hand, crushing is visible in the medullary ray parenchyma from the first. Moreover, the new cells have the same diameter (radially) as the cambium cells, and for the first few divisions their longer axes are in a line with the long axes of the cambial cells (as seen in transverse section). The phloem and phloem fibres show displacement, and there are more cells in the cambial layer at the point where the root initial is than at the other side of the bundle. It seems therefor a legitimate conclusion that the new meristem is produced by the cambium of the vascular bundle. The first divisions of the cambium are apparently tangential, and then the smaller, nearly isodiametric cells of the root initial are produced by

radio-longitudinal and transverse divisions.

The established root-initial is quite unmistakable by reason of its intensely dense cytoplasm. It is evident that active protoplasmic synthesis must be taking place, and the diminishing starch in the adjacent cells shows that carbohydrate is involved. It is significant in this connection that all the roots so far examined, without exception, are so closely connected with a vascular bundle. This is in contrast to callus, which is initiated, as described above, in the medullary rays. The suggestion of Professor Dixon (7) that the phloem is in the nature of a store-house of enzymes may have some bearing on this question, but the true function of the phloem is still too vague for any decision.

The axis of the root-initial soon follows a radius of the stem, and growth proceeds through the medullary ray. (Fig. 36). This is no doubt purely a mechanical question. At first simply a cone of embryonic cells, the root differentiates very soon. The first stage is the appearance of a core of elongated, vacuolated cells (beginning of plerome). Then an outer layer of slightly larger cells is distinguishable, and by the time the tip breaks the epidermis the tissues are fully mapped out. The nuclei are very large ($2-2.5\mu$), and spherical, not discoid as so often in the cambial cells. The growing organ pushes its way through the cortical cells, which are seen crushed around it. By this time the cells at the base of the root have acquired the direct cellulose

reaction.

The first definitely vascular elements connecting the stem and root supply are laid down in the neutral territory at the base of the root, in fact by the remains of the cambial zone from which the root originated. These first-formed elements are small tracheids, which become lignified and pitted. The protoxylem elements which then differentiate in the plerome are directly connected with the small tracheids just described: the sloping terminal wall of the vessel being apposed to one face of such a cell. Connection with the stem bundles is at first of a slight description, since these first-formed elements at the base of the root do not necessarily link up at once or completely. As growth proceeds, the tissues of the stem are invaded by a mass of secondary tracheids, which unite with one or both of the bundles adjoining the ray through which the root emerged. (Figs. 37,38). The cambial cylinders of the root and stem are in continuity, and probably both contribute to the secondary wood at the juncture of stem and root. How far the meristem which spans the base of the root is active is difficult to decide, but it seems to be responsible for the development of a transverse bar of cross-grained wood above and below the core of wood proceeding to the adventitious root. (Fig.38,B). The root acts as a wedge, forcing the ray apart, and it seems likely that this cross-graining is partly a mechanical response to this disruption. It is cer-

certainly part of the response to cutting, as it is never seen in a normal stem. In any case, adventitious roots are quite efficient enough to allow of as full a development of the plant from a cutting as from a seedling with a tap-root.

C. EXPERIMENTS IN PROPAGATION.

C. EXPERIMENTS IN PROPAGATION.

It was found that, by etiolating the stem before taking the cutting, rooting would take place readily from the node. A length of stem of suitable age and size was selected, and the leaves removed from two or three (or more) successive nodes. The stem was then wound with strips of black paper, fixed with a light tie of raffia. The removal of the leaves and the darkening of the stem acted in conjunction to deplete the carbohydrate reserves in that particular region of the plant, while the water-supply was not interfered with. After periods of time varying from eighteen to twenty-eight days the dark paper was removed, and the cuttings made. Usually half the number were made at the internode as a control. It was found that the partial blanching, besides making it possible for rooting to take place from the node, hastened the rate of rooting from the internode. (Figs.39). If, however, the etiolation had been carried on too long, the cuttings were weakened and soon died off. The best time for any species must be found by trial, as they differ considerably in this respect.

Painting the stem with Indian ink was also tried, but is not to be recommended. A cutting of C.smilacifolia treated in this way rooted freely from the node, but subsequently growth was exceedingly slow, as the Indian ink remained on the stem and thus prevented normal assimilation. (Fig.41).

An interesting case of a natural rooting at the node was that of a six year old stem of Clematis montana, which had been lying on the ground in partial shade. The stem was rooting freely at the node and not at the internode. (Fig.43). By examining the roots it was seen that the oldest root was five years, so that the stem must have been prostrate for that length of time, and have rooted in its first year.

A comparison of the etiolated with the normal stem showed a great decrease in the amount of starch present. The xylem parenchyma, the pith and the medullary rays were practically starch-free: the starch-sheath was more prominent even than usual, and the amount of starch in the cortex was greatly reduced.

After etiolation the pericyclic fibres and the pith stained much less strongly than before with Phloroglucin and HCl. (Fig.42). The walls of the fibres were also reduced in thickness. (In one example of C.Forestii, the normal fibre wall was 8μ thick: after etiolating for 28 days, the fibre walls were 5μ thick, in each case average of 20 readings). In normal stems of an age suitable for cuttings there was always some starch in the pericyclic fibres and a great deal in the pith: that is, these cells, in spite of their thickened walls, must be considered to be alive, and therefor it is questionable whether they can be regarded as truly lignified, in spite of their positive reaction with

Phloroglucin-HCl. There can be no doubt, however, that the process of polysaccharide deposition on a cell-wall is reversible so long as the protoplast remains alive, and that one means of reversing the reaction is by etiolation.

The production of callus, roots and shoots by an isolated portion of a plant is a process of regeneration which depends primarily upon the continuation of meristematic activity by those tissues of the plant which have remained meristematic (e.g. cambium), or by the resumption of the embryonic state by mature tissues. The process of cell-division is the expression of a series of catenary reactions, depending upon the presence in a certain concentration and a certain ratio of carbohydrate and amino-radicles. Only when this carbon-nitrogen balance is maintained can a cell remain meristematic. Under normal conditions (of illumination etc.) the carbohydrate is always in excess, and this excess is one of the factors which determine cell-maturity and the cessation of new growth. If by any means the required C:N ratio can be restored, a mature tissue can be incited to regeneration. Etiolation of the part in question while still attached to the parent plant and in full communication with the vascular supply should, theoretically, act in this way, and this is confirmed by experiment.

The anatomical structure of the node of Clematis shows that it presents mechanical difficulties in the way of rooting. There is a large amount of schlerenchyma and very little cambium. It is evident that etiolation acts in two

ways: firstly, by exciting meristematic activity, and secondly by "softening" the hard tissues of the fibres and pith. In this way the resistance of the tissues to the emergent root is lessened, and rooting proceeds freely from the node.

SUMMARY.

1. The genus shows a fundamentally hexagonal stem with a simple vascular pattern based upon 6 or 12 primary foliar bundles. Secondary bundles may be added by the interfascicular cambium, culminating in the type with a ring of many bundles.
The leaf-trace strands run through one (6-bundled stems) or two (12-bundled stems, many-bundled stems) internodes before losing their identity.
 2. No continuous cylinder of wood is formed, the xylem-mass being divided by the persistent primary rays into segments corresponding to the original bundles. The wood consists of large pitted vessels and tracheids.
 3. The first periderm is deep-seated, arising in the pericycle. Succeeding cork-cambiums appear in the phloem, giving the characteristic string-bark of the genus.
 4. The first signs of callus formation consequent upon vegetative propagation is an abnormal activity of the interfascicular cambium.
 5. Adventitious roots originate from the cambium of a vascular bundle, and grow out through an medullary ray. The vascular supply of the root is connected to a stem bundle by a mass of secondary tracheids developed at the base of the root.
 6. Previous etiolation of a stem enables rooting to take place at the node. An etiolated stem shows diminution of carbohydrate content, and altered reactions of the cell-walls of the fibres and pith.
-

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LIST OF MICROCHEMICAL REAGENTS, STAINS ETC.Lignin.

- 1). Phloroglucin, 5% in 96% Alcohol, Conc. HCl.
 (Chamberlain, "Methods in Plant Histology" 3rd. ed.)

Lignin and Cellulose.

- 2). Iodine.....1 part } Zinc chloride ..2 parts } B
 Potassium iodide..1 part } A. Water.....1 part. }
 Water.....100 parts.)

Mount the section in equal parts A and B.

(Artschwager, Ernst. " Use of Chloriodide of Zinc in Plant Histology." Bot. Gazette, 71:400.(1921)).

Suberin.

- 3). Sudan III.....0.1g.
 Glycerine.....50cc.
 Abs. Alcohol..50cc.
 (Kroemer, Karl. " Wurzelhaut, Hypodermis und Endodermis der angiospermwurzel." Bibliotheca Botanica, 59.1903).

Blés Fixing Fluid.

Absolute alcohol.....6 parts.
 Formalin.....3 parts.
 Glacial Acetic Acid....1 part.

Safranin, 1% in distilled water.

Gentian Violet, 1% in distilled water.

Light Green, saturated solution in clove oil.

Orange G, 1% in clove oil.

LIST OF ILLUSTRATIONS.

Fig.	SUBJECT.	P.	Fig.	SUBJECT.	
1.	<u>C.coccinea</u> , T.S.Stem.	55	23.	<u>C.montana</u> , T.S.Periderm.	84
	<u>C.patens</u> , " "				
2.	<u>C.integrifolia</u> , " "	56	24.	Immature Xylem & Phloem.	86
	<u>C.eriostema</u> , " "		25.	<u>C.montana</u> , T.S.secondary wood.	88
3.	<u>C.marata</u> , " "	57			
	<u>C.viticella</u> , " "		26.	R.L.S.Wide Ray and Pith.	88
4.	<u>C.virginiana</u> , " "	58	27.	Details of Ray Cells.	87
	<u>C.Vitalba</u> , " "		28.	Details of Secondary Wood.	89
5.	<u>C.ranunculoides</u> , " "	59	29.	" " "	90
	<u>C.afoliata</u> , " "				
6.	<u>C.orientalis</u> , " "	60	30.	<u>C.smilacifolia</u> , L.S.Wood.	92
	<u>C.napaulensis</u> , " "		31.	Cuttings.	93
7.	<u>C.smilacifolia</u> , " "	61	32.	<u>C.smilacifolia</u> , early callus. T.S.	94
	<u>C.heraclaeifolia</u> , " "				
7A.	Diagram, shapes of stem.	62	33.	" " L.S.early callus.	96
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9.	Diagram, 6-bundled stem.	63	35.	<u>C.gracifolia</u> , L.S.older callus.	98
10.	Many-bundled stem.	65			
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20.	<u>C.afoliata</u> , sunken stoma.	81			
21.	<u>C.uncinata</u> , L.S.apex.	82			
22.	<u>C.heraclaeifolia</u> , starch-sheath.	83			

Figures 1-7.

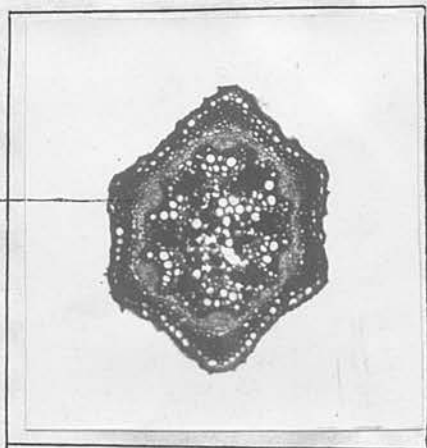
Transverse sections of stems of Clematis, all in their first year of growth, to show the more common types of stem and some of the extremes.

Six stems with six bundles, six with twelve bundles, and two with many bundles, have been chosen for illustration.

All the sections are hand cut, treated with Phloroglucin-HCl-Iodine (hereafter referred to in the legends as P.H.I.), and mounted in glycerine. The photographs are on Eastman Commercial Ortho Film, taken with a green screen (Wratten "B" filter) in order to emphasize the vascular structures.

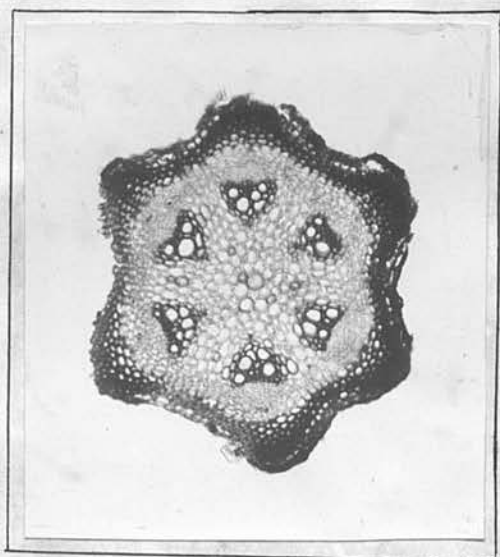
Figure 1.

periderm

C. coccinea x Scottii.

x 54

x 54

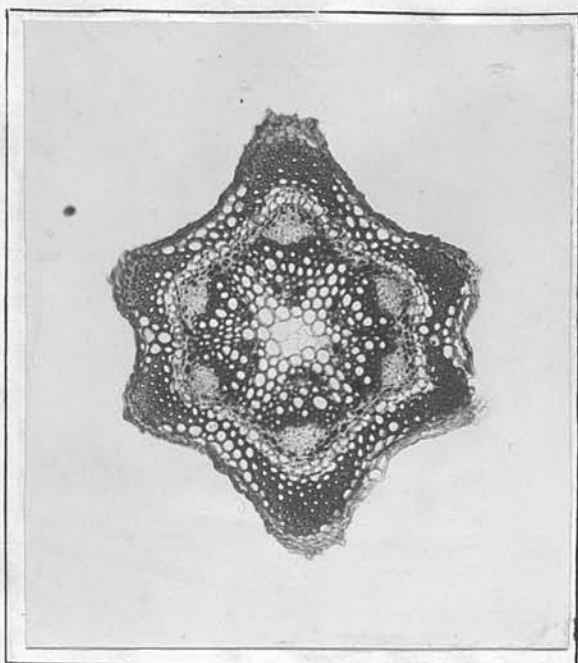
G. patens.

x 54.

x 54

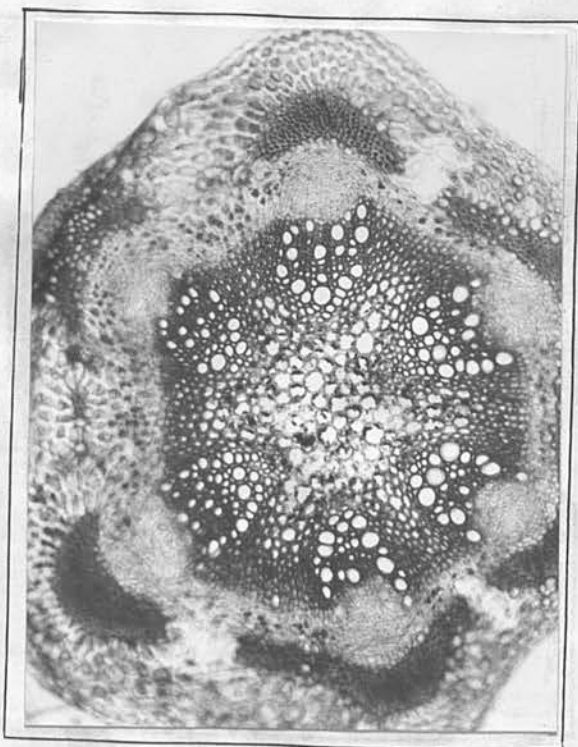
Figure 2.

57 56.



C. integrifolia
x villida.

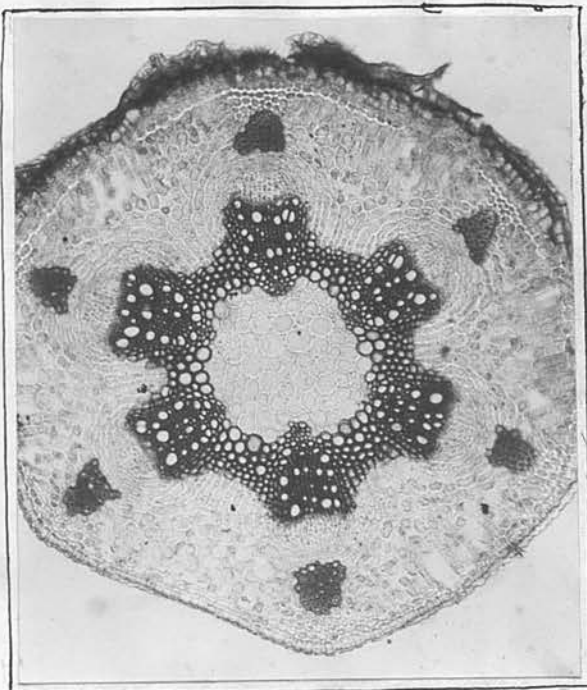
x 54



C. eriostema.

x 54.

periderm

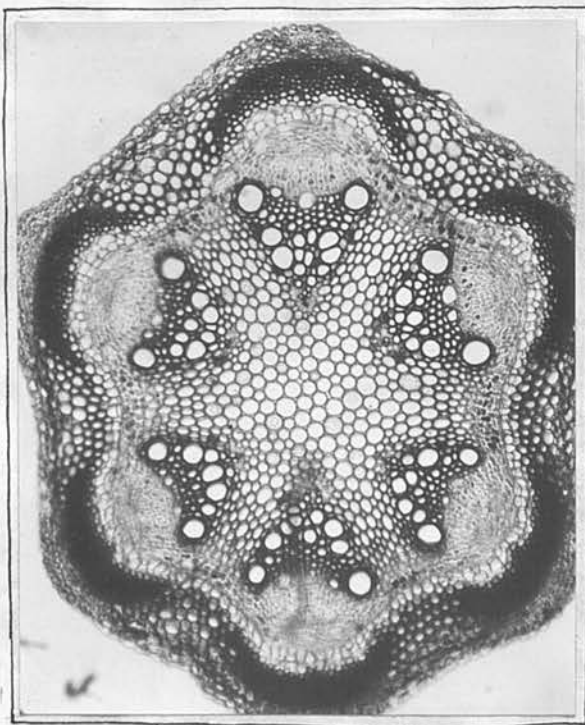


C. marata . x 54.

C. virginiana

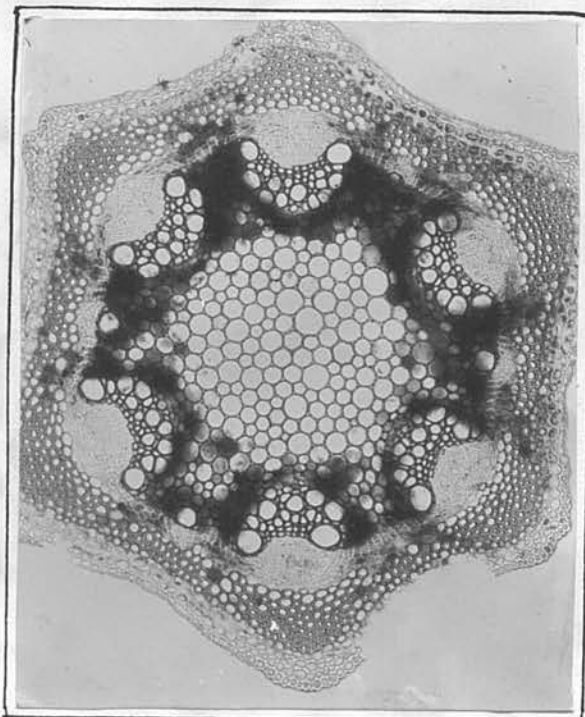
x 77.

periderm



C. viticella .

x 54.

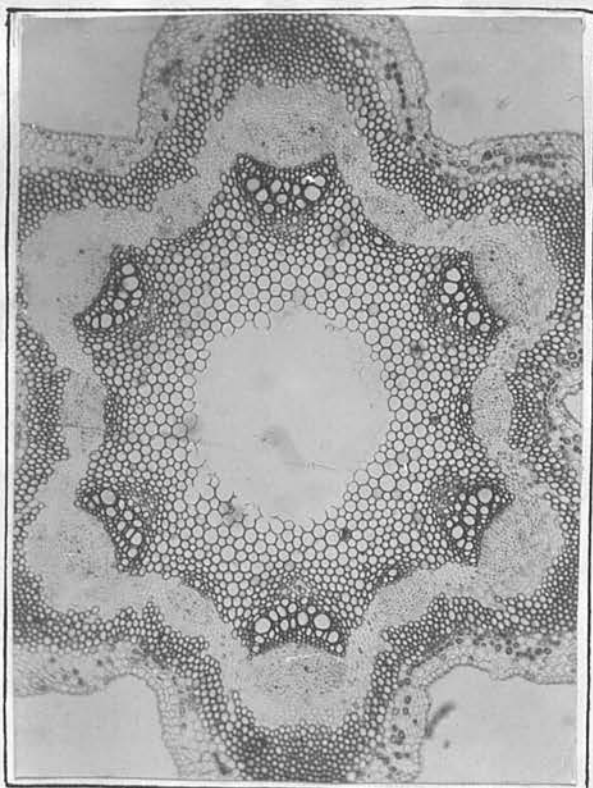


C. ramunculoides.

x 18

C. virginiana

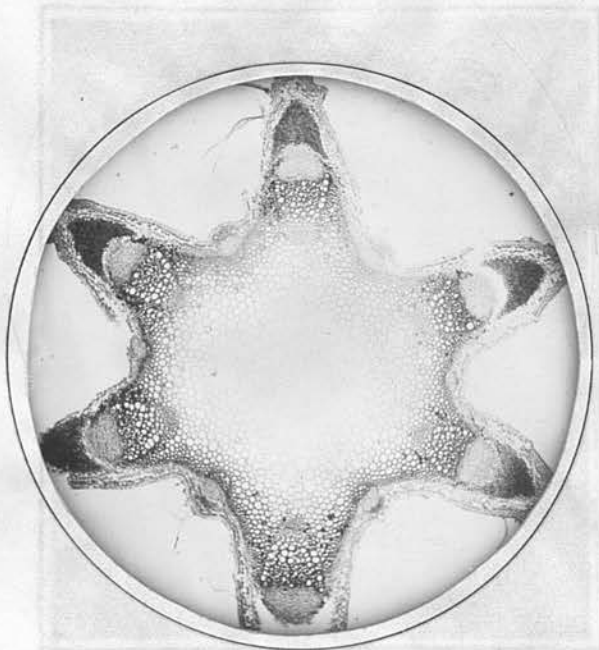
x 77.



C. Vitalba.

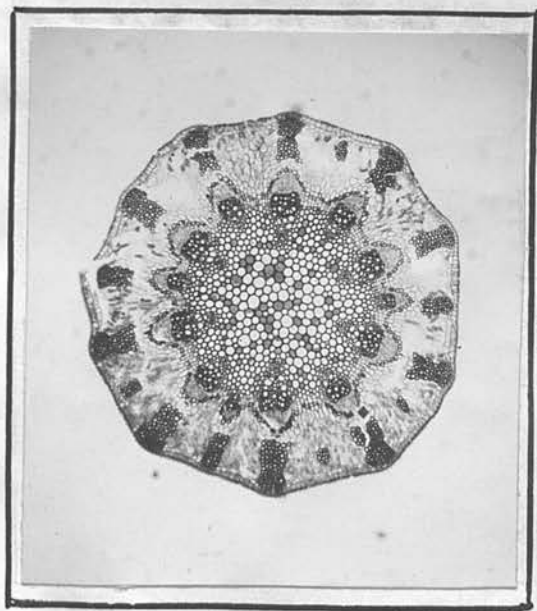
x 54.

Figure 5.



C. ranunculoides.

C. x 18 ~~alis~~ x 28



C. afoliata

x 28.

Figure 6.

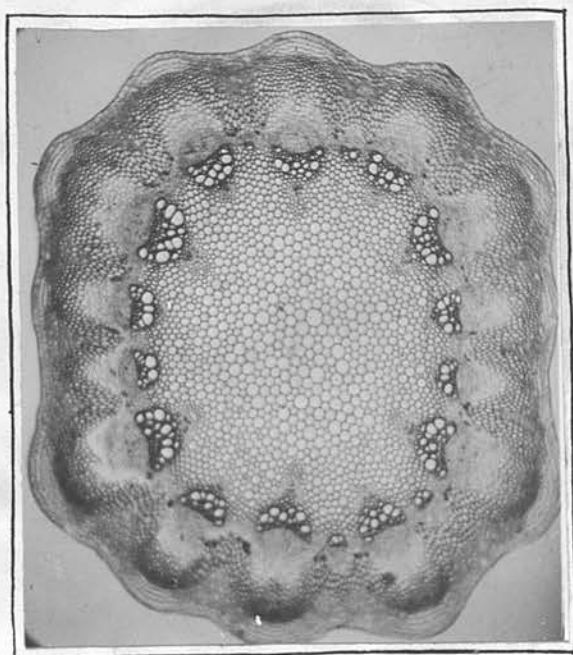
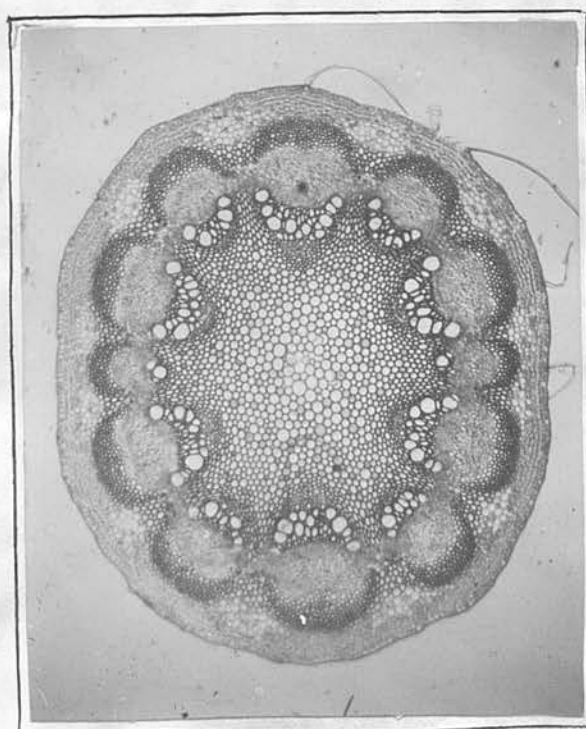
C.orientalis . x 28C.napaulensis. x 28heraultensis. x 18.

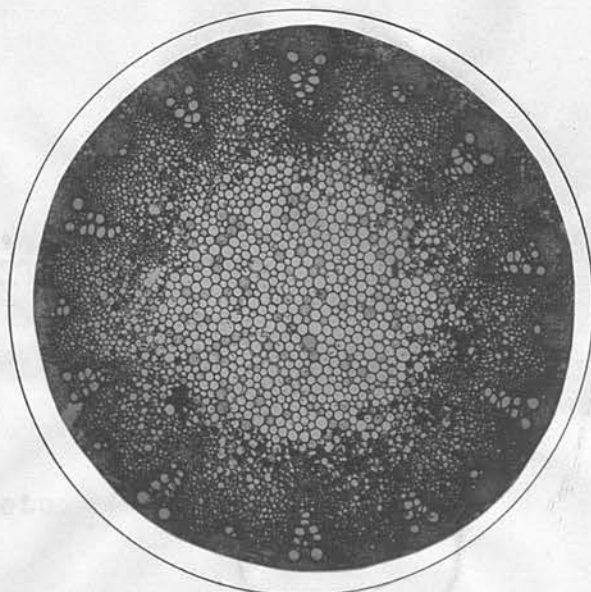
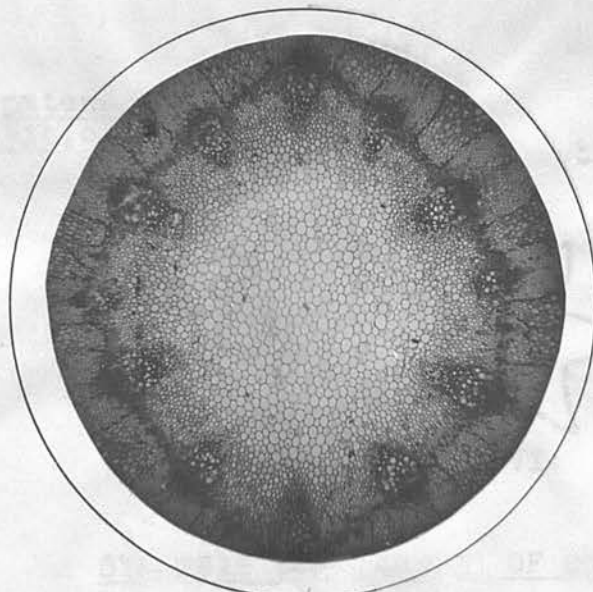
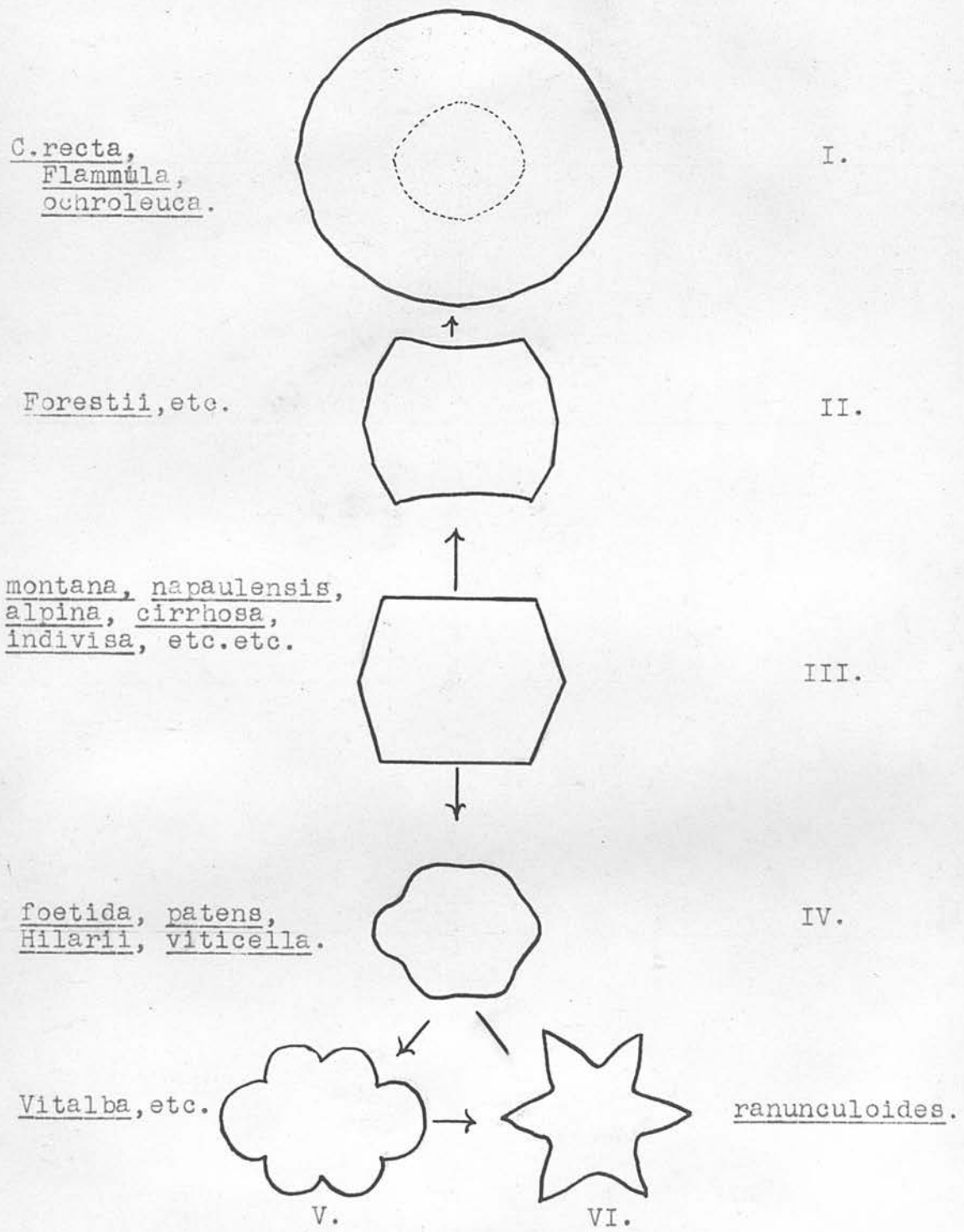
Figure 7.C. Smilacifolia. x 18.C. heraclaeefolia. x 18.

Figure 7A.



SYNOPSIS OF SHAPES OF STEM
IN CLEMATIS.

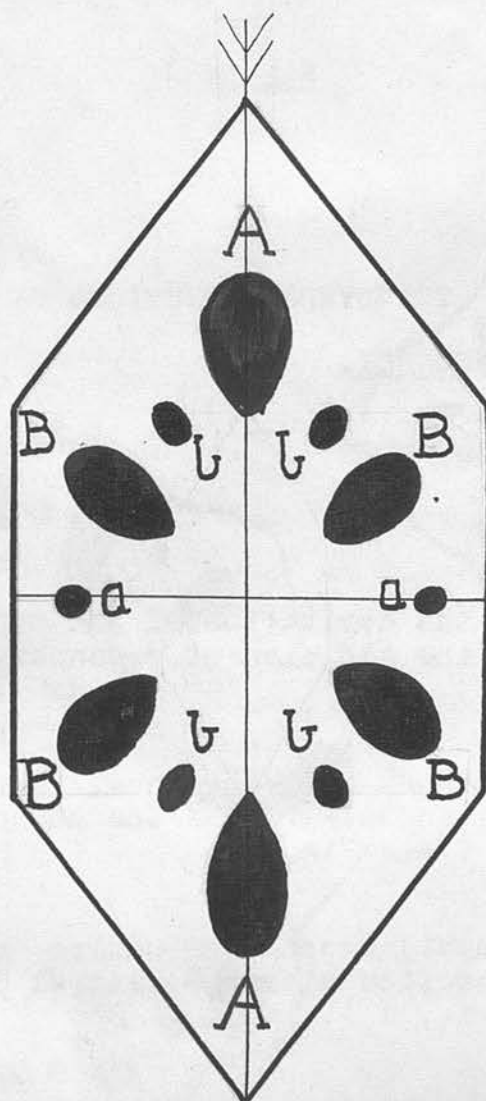


Figure 8.

12-bundle stem.

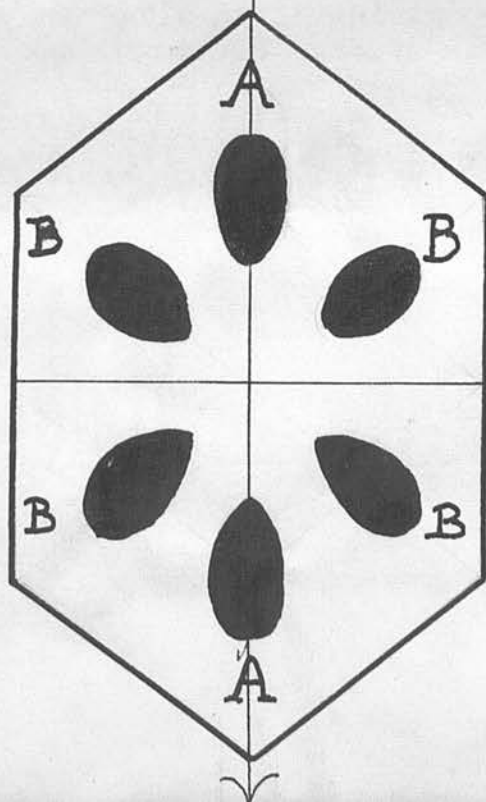


Figure 9.

6-bundle stem.

Figure 10.

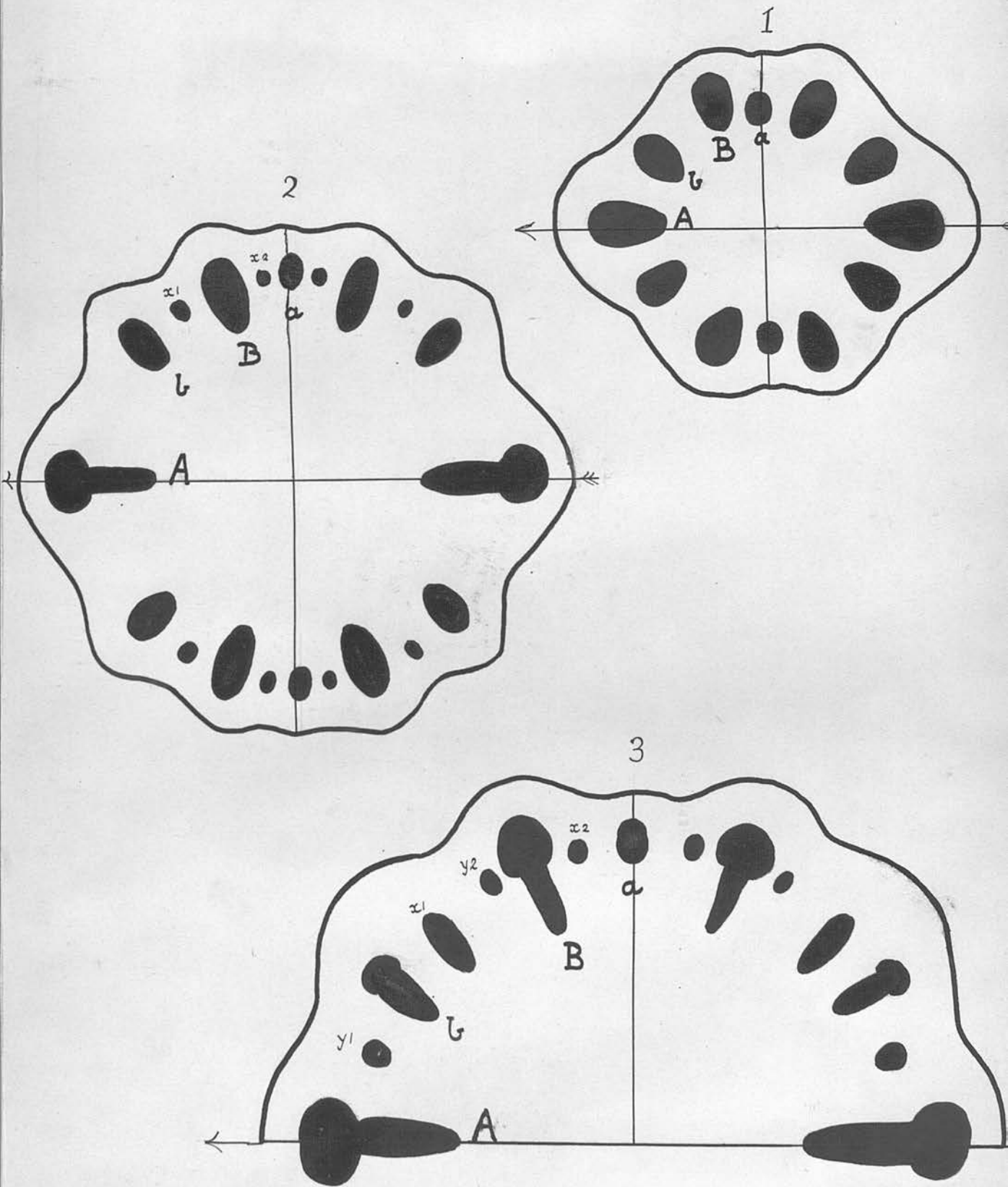


Figure 10.

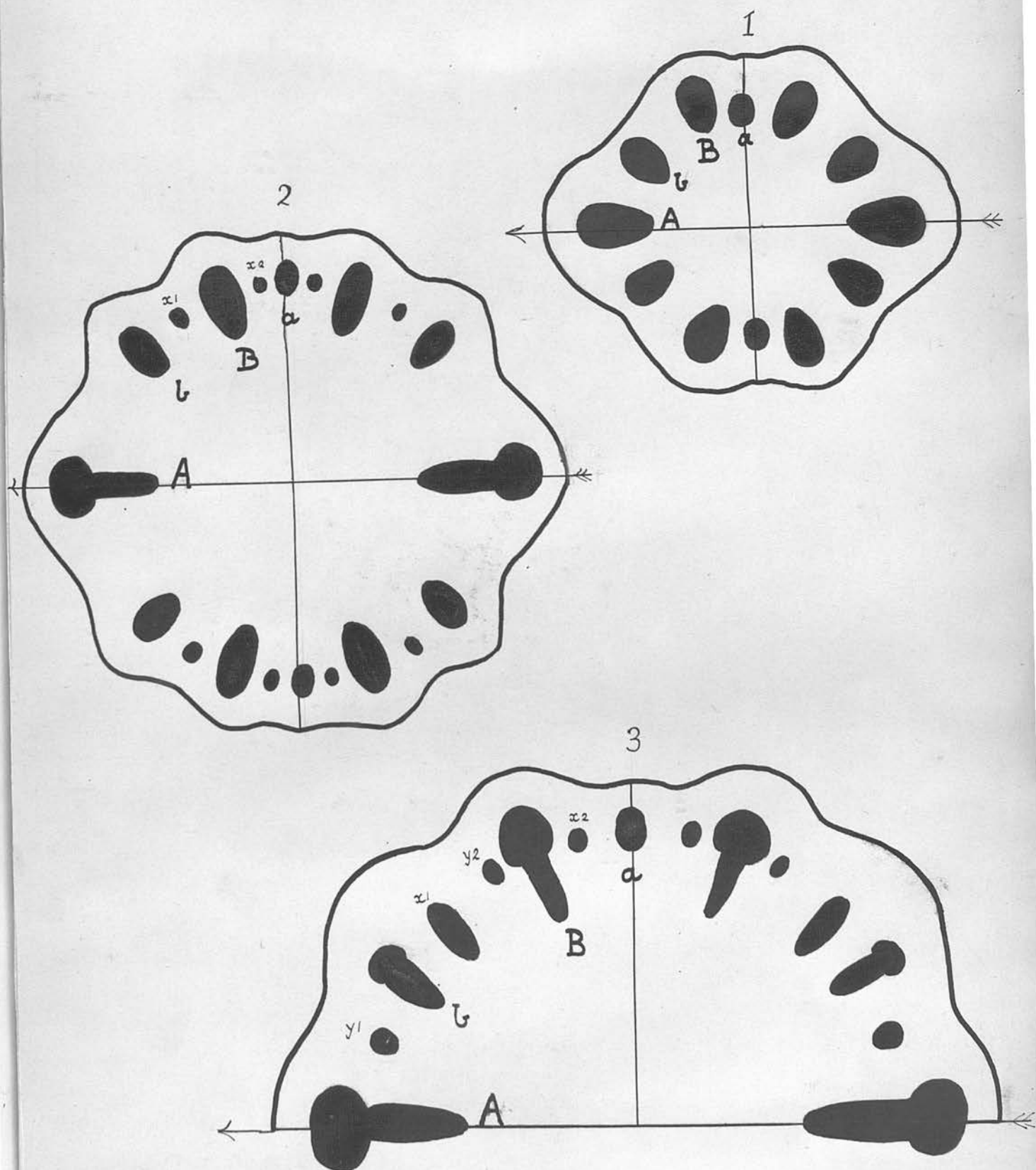
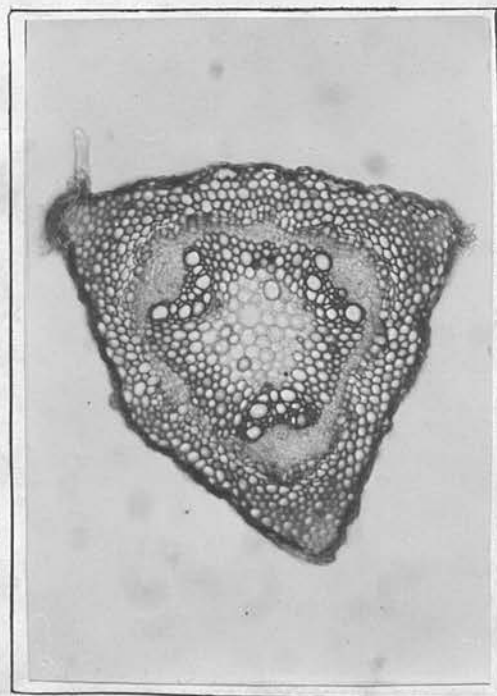


Figure 12.
Figure 11.

TRANSVERSE SECTION OF THE ROOT OF CLEMATIS INTEGRIFOLIA.
 TRANSVERSE SECTION OF THE STEM OF CLEMATIS YUNNANENSIS.



epidermis

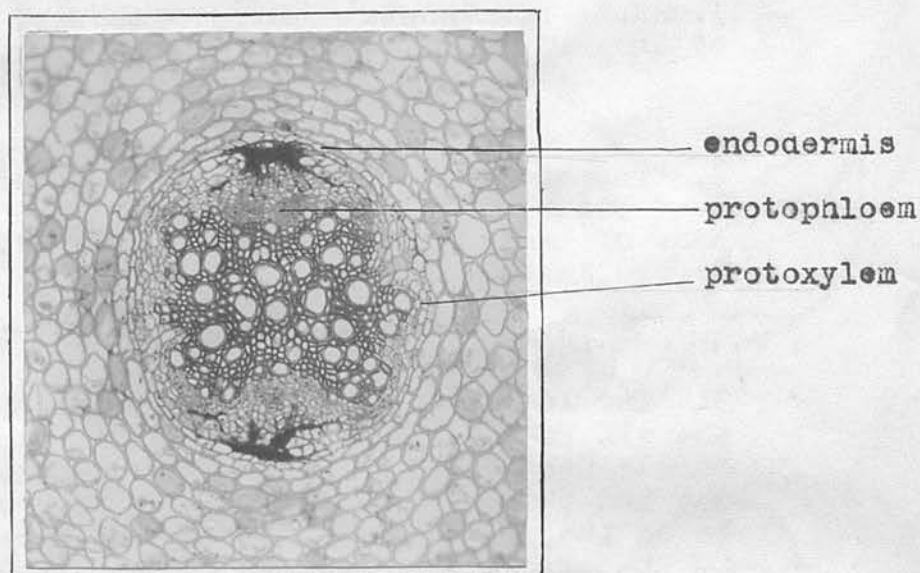
phloem

protoxylem

This section shows a well-marked epidermis with radial dots, the xylem in the form of a clear plate, and the first beginning of secondary thickening. This section is a seedling plant showing a juvenile transition stage from alternate to opposite leaves. and anilin blue. There are six bundles present in the stem, but three are much better developed than the others, and the stem is approximately triangular in section.

Figure 12.

TRANSVERSE SECTION OF THE ROOT OF CLEMATIS INTEGRIFOLIA.
x 77



This section shows a well-marked endodermis with radial dot, the xylem in the form of a diarch plate, and the first beginnings of secondary thickening. Much starch is stored in the cortex.

Hand-cut section, stained safranin and anilin blue.
Negative on E.C.Ortho Film, Wratten "B" filter.

Figure 13.

SERIES OF TRANSVERSE SECTIONS THROUGH THE
NODE OF CLEMATIS PATENS. x45.

(From camera lucida drawings).

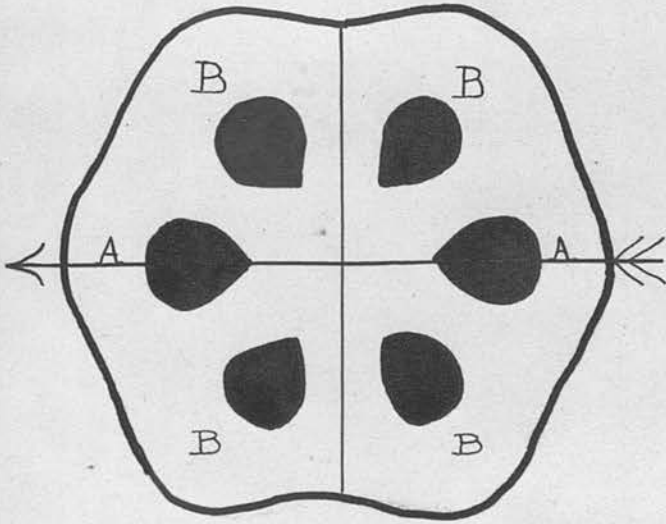
1. This form shows a simple vascular pattern of only six bundles. (2A+4B).

2. The stem is practically surrounded by the leaf bases, which are still free from it. The base of the bud shows a ring of bundles, derived directly from B,B. (see3).

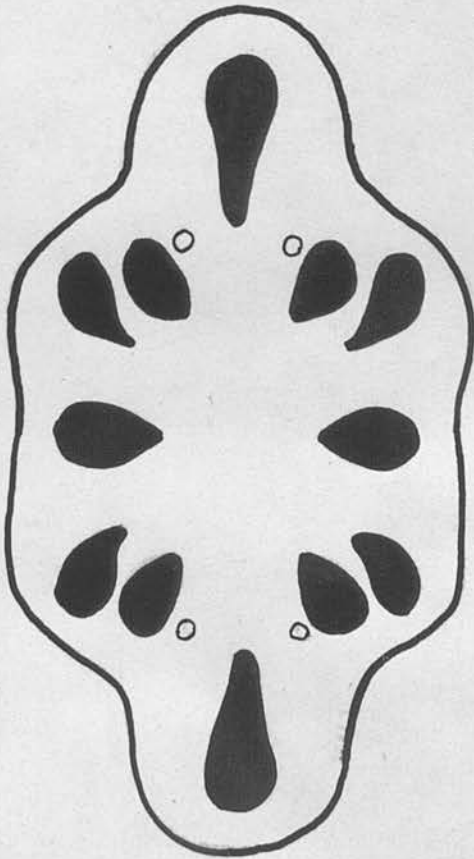
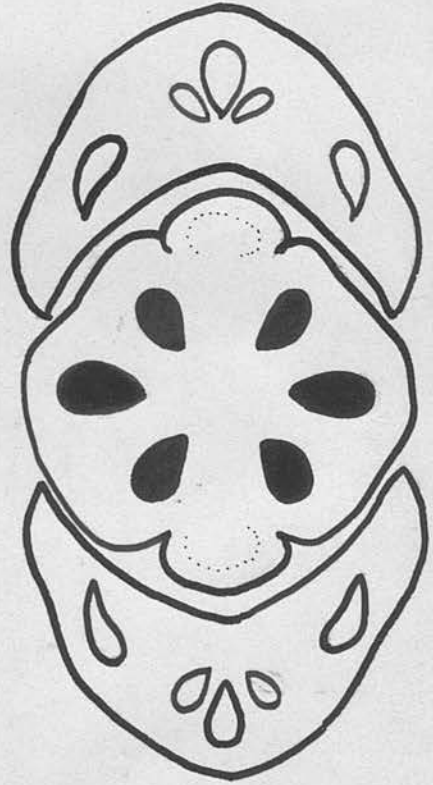
3. The leaf-bases have fused with the stem, and the six leaf-trace strands are approaching the bundle ring. The pairs of bundles which supply the bud (drawn with a line only) are seen to be on the point of fusing with B,B.

4. The A and B bundles divide into two, and fuse straightway with the nearest leaf-trace strands. Thus the median leaf-trace strand fuses on each side with one-half B: the lateral leaf-trace strands receive one-half B on one side and one-half A on the other. In this way the six bundles of the internode are directly reconstituted.

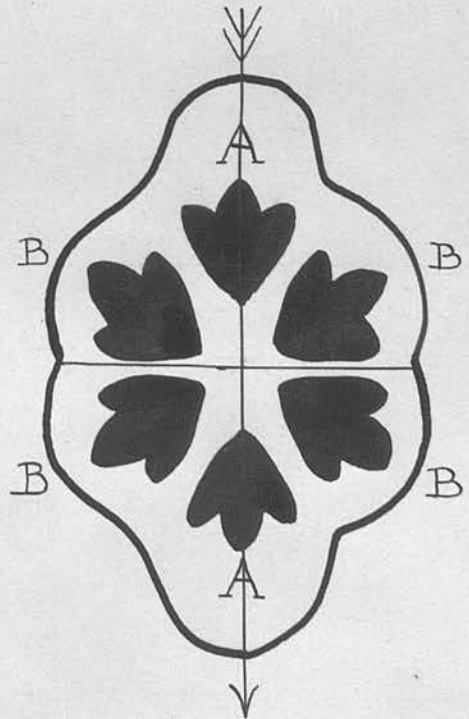
1



2



3



4

Figure 14.LONGITUDINAL PLAN OF THE COURSE OF THE BUNDLES
THROUGH THE NODE OF CLEMATIS PATENS.

Constructed from a series of camera lucida drawings. Vertical scale slightly exaggerated. Numbered and lettered to correspond with the series of transverse sections in Figure 13. The whole stem is shown in plan.

Simple vascular pattern of six bundles only. (2A+4B). Two successive nodes are shown, the internode much condensed and the strands drawn in outline only. The entering leaf-trace strands are drawn with a broken line until they are in the same vertical plane as the bundle ring. In this form the leaf-trace strands pass through one internode only before dividing and fusing with the corresponding strands in the node below.

Fig. 14.

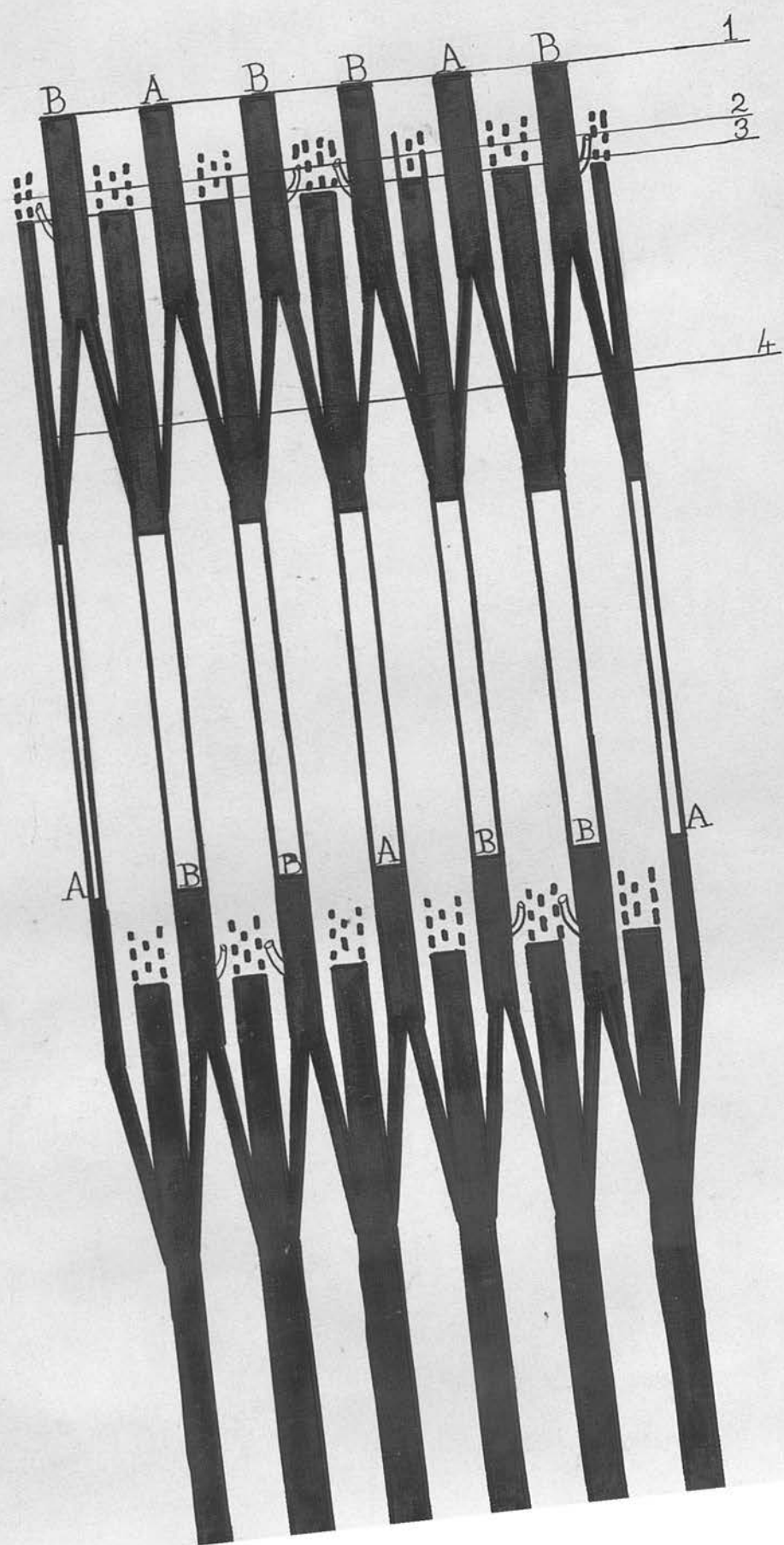


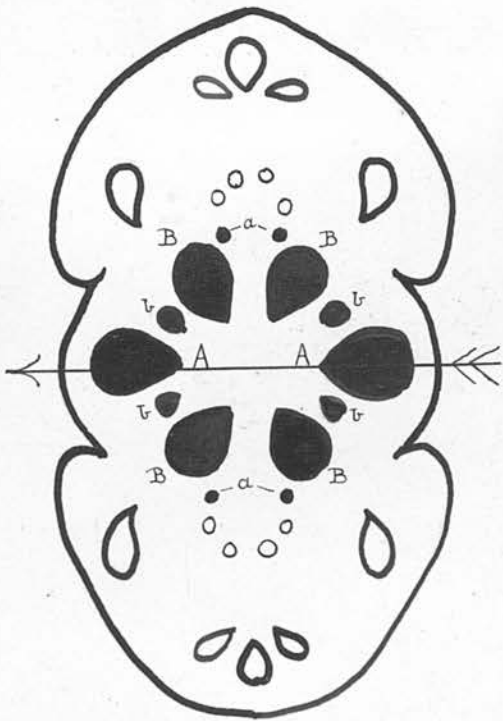
Figure 15.

SERIES OF TRANSVERSE SECTIONS THROUGH THE
 NODE OF CLEMATIS VITALBA, x 22.5.

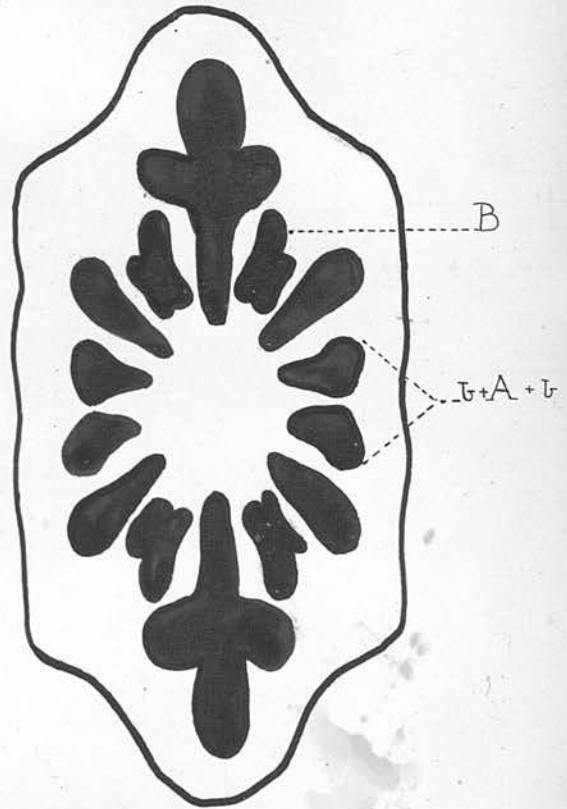
Reduced from camera lucida drawings.

1. The leaf-bases are not yet completely fused with the stem, and each shows the three trace-bundles, the three components of the median bundle being still distinct. The ring of bundles at the base of the bud is derived from the a-bundle on each side. The long axis of symmetry of the internode above is shown by an arrow.
2. The leaf-bases are now completely fused with the stem. The A-bundles have fused with the b-bundles and then split into two, while the halves of a have fused with B. This fusion-bundle is also about to divide into two. The six leaf-trace strands have entered the bundle-ring; the median strands between B, B, the laterals between B and A.
3. The halves of A each give a small strand from their apposed sides: these fuse, re-forming the a-bundle of the next internode. The remainder of A fuses with the lateral leaf-trace bundle. The halves of B behave similarly. The median leaf-trace bundle thus fuses with a portion of the B-strand on each side, giving the A-bundle of the next internode, while the lateral leaf-trace bundles receive a portion of B on one side and A on the other, and give the new B-bundles.
4. With the completion of these fusions, the typical 12-strand arrangement of bundles has been restored, with the long axis of symmetry at right angles to that of the internode above.

1



2



3

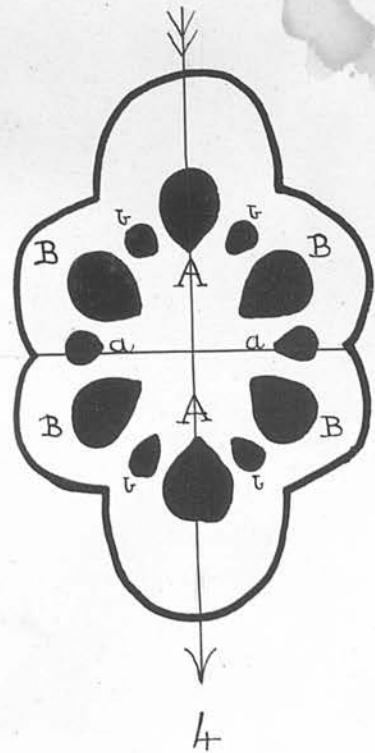
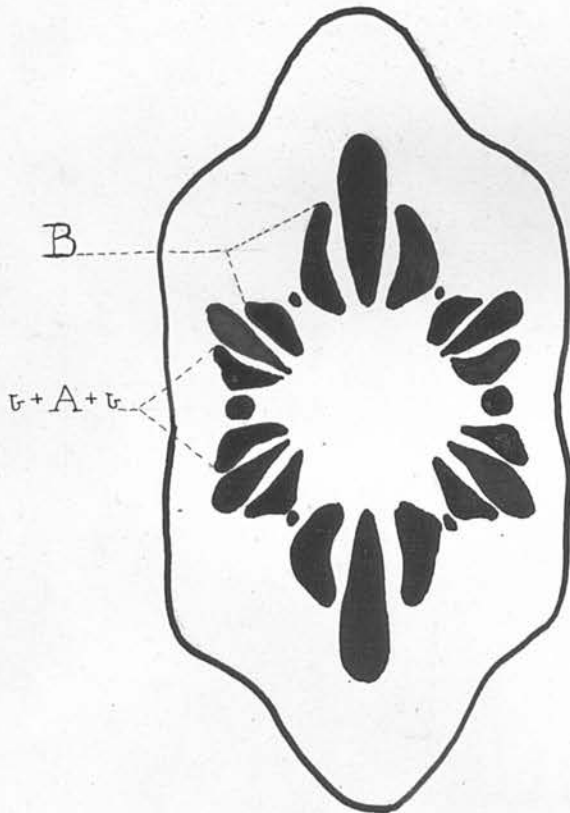


Figure 16.

LONGITUDINAL PLAN OF THE COURSE OF THE BUNDLES
THROUGH THE NODE OF CLEMATIS VITALBA.

Constructed from a series of camera lucida drawings, the vertical scale exaggerated for the sake of clearness. Numbered and lettered to correspond with the transverse sections in Fig. 15.

The leaf-trace bundles entering the node are drawn with a dotted line until they are in the same vertical plane as the bundle-ring. One-half only of the stem is shown.

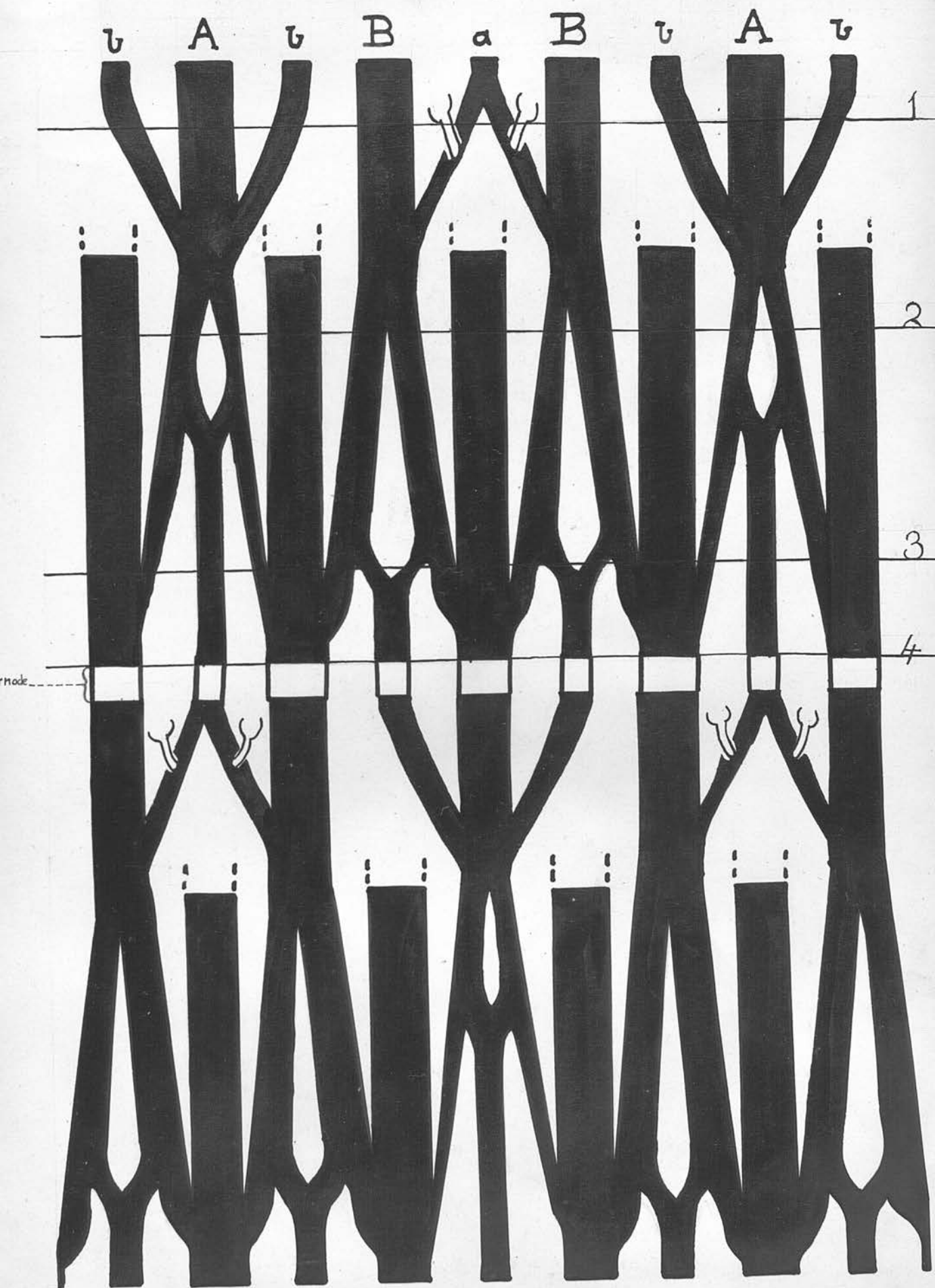


Figure 17.

SERIES OF TRANSVERSE SECTIONS THROUGH THE
NODE OF CLEMATIS SMILACIFOLIA.

Reduced from camera lucida drawings.

1 and 2, x 25: 3 and 4, x 50.

1. This form shows a more complicated vascular pattern of 16 bundles, ($2(A+2)+4B+2a+4b$). At this stage the buds are partly fused with the stem, while the leaf-bases are still free. The ring of eight bundles at the base of the bud is derived from the a-bundle after it has divided in two.

2. The leaf-bases are completely fused with the stem, and the six leaf-trace strands are approaching the bundle-ring, the median strand penetrating between the halves of a, and the lateral strands between the halves of b.

3. One-half of the stem is drawn on a larger scale to show the stage of greatest dispersion of the bundles. A has fused with its auxiliaries on either side, and the resulting fusion bundle has divided into two, each half then giving a small strand which unites with its neighbour to re-form the a bundle of the next internode. The halves of a are just beginning to divide again, to give on the one hand the auxiliary bundles to the median leaf-trace strand, and on the other hand a small portion which will join up with B.

4. Coalescence of the scattered strands to form the sixteen bundles of the internode. Thus:-

- A is the median leaf-trace strand + 2 auxiliaries.
- b " $a/2 + B + b/2$.
- B " the lateral leaf-trace strand, + $b/2 + A$ etc $a/2$.
- a " a portion of $A + 2$ aux.

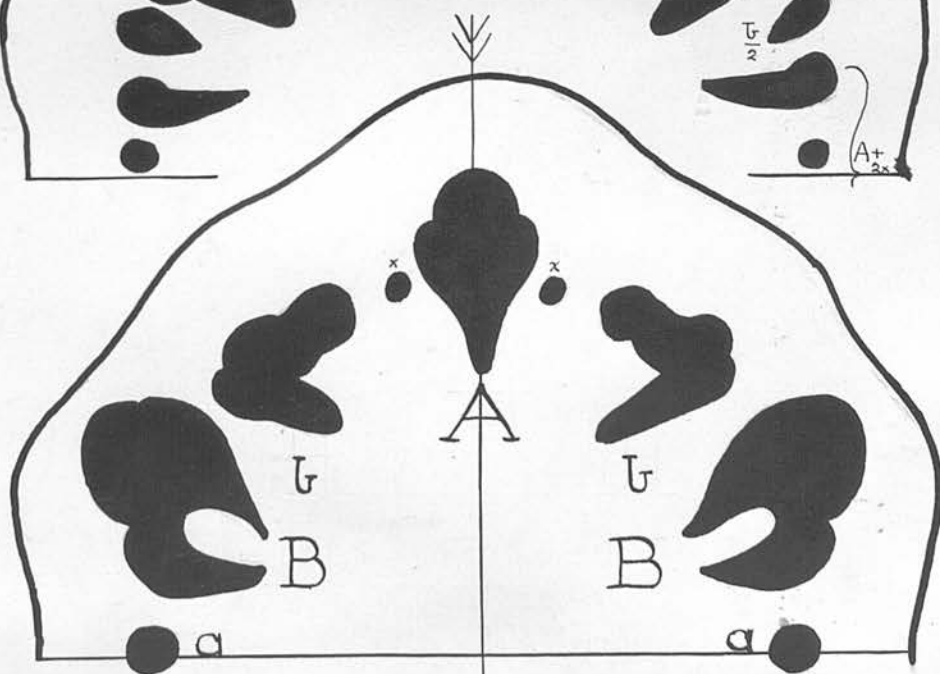
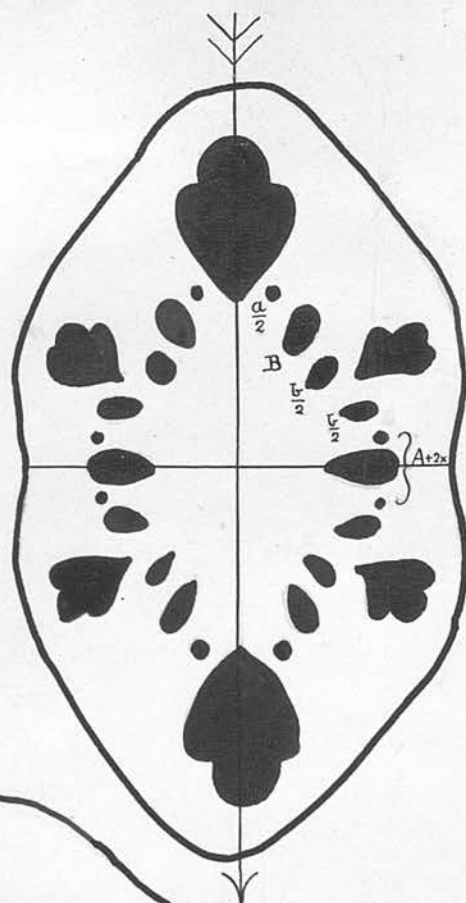
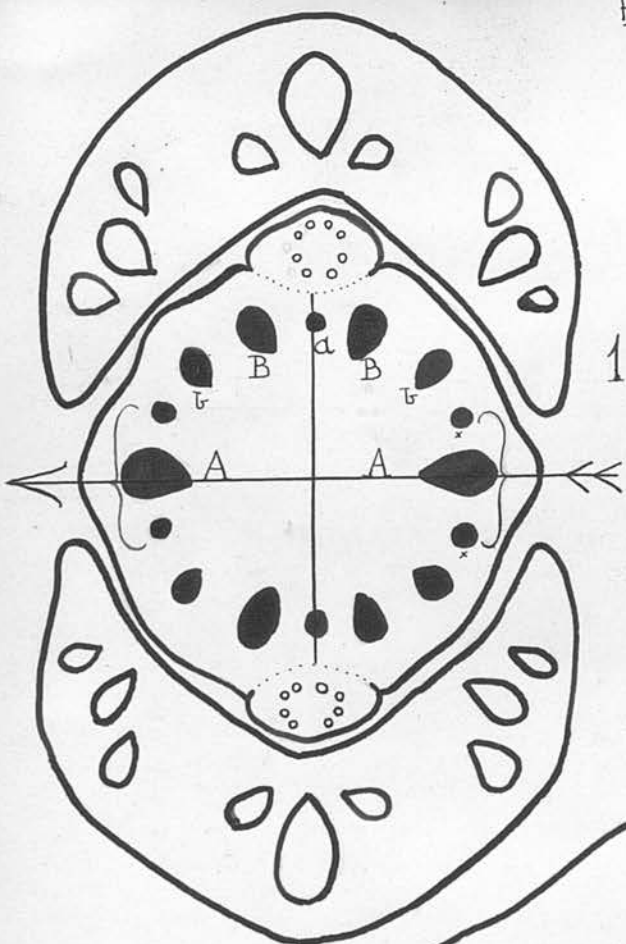


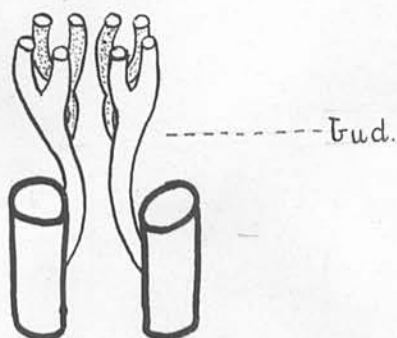
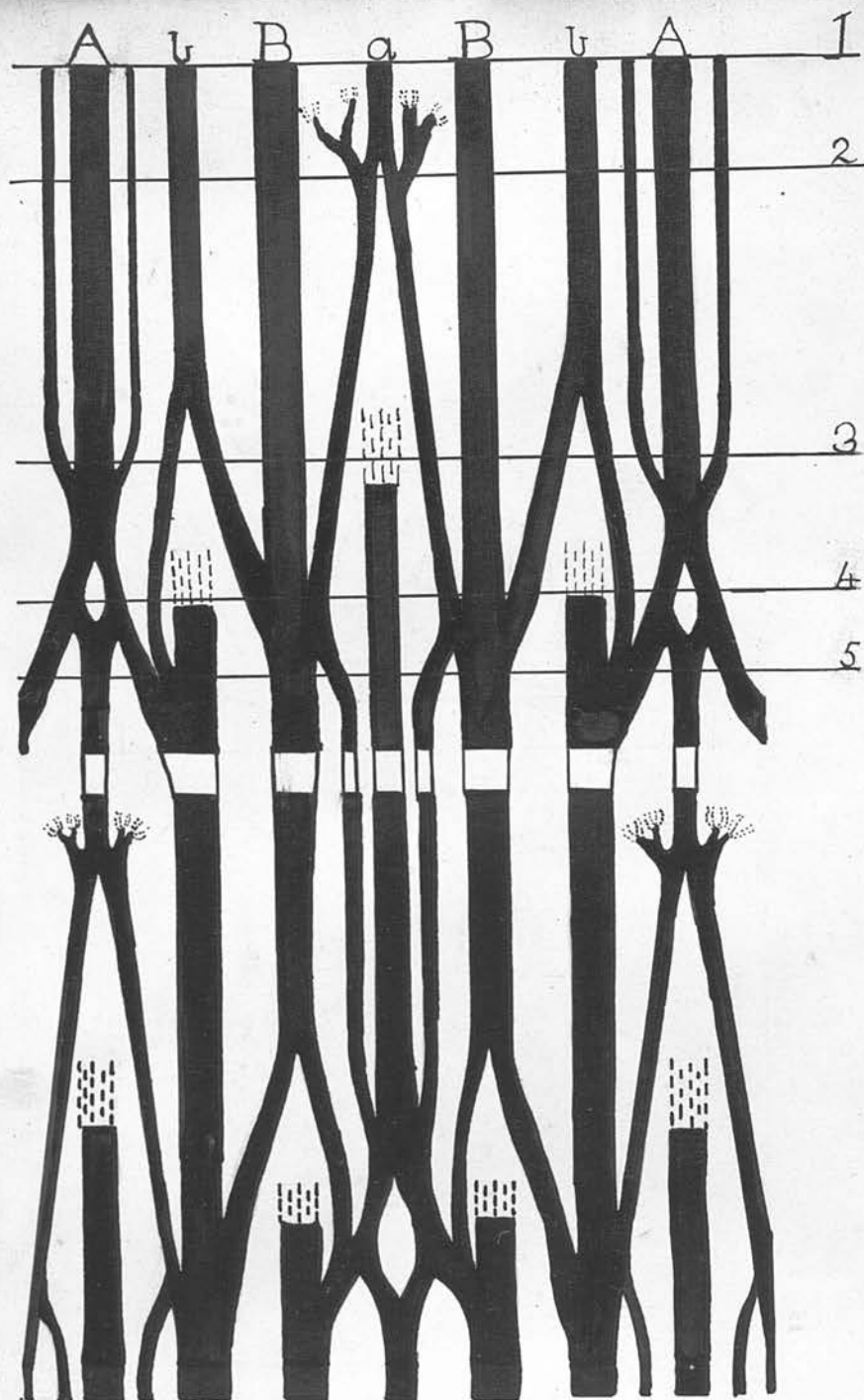
Figure 18.

LONGITUDINAL PLAN OF THE COURSE OF THE BUNDLES
THROUGH THE NODE OF CLEMATIS SMILACIFOLIA.

Constructed from a series of camera lucida drawings, the vertical scale exaggerated for the sake of clearness. Numbered and lettered to correspond with the transverse sections in Fig. 17.

Two successive nodes are shown, the internode much condensed and drawn with a line only.

The bundles supply to the bud is drawn below on a larger scale.

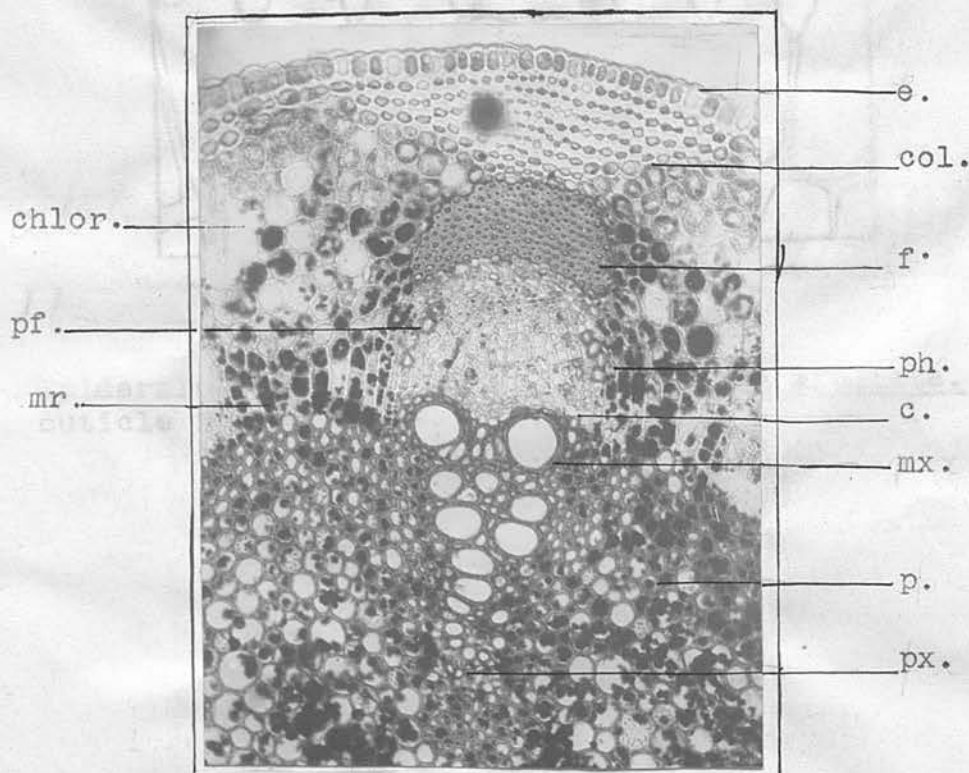


bud.

Figure 19.

TRANSVERSE SECTION OF THE STEM OF CLEMATIS SMILACIFOLIA.

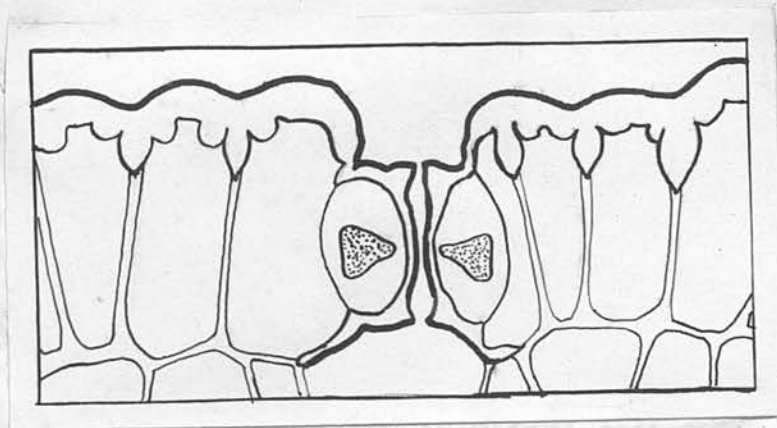
Hand section, mounted P.H.I. Negative on E.C.Ortho film, Wratten "B" filter. x 77.



- | | |
|--------------------------|-------------------------------|
| e.....epidermis | c.....cambium |
| col....collenchyma | mx.....large metaxylem vessel |
| chlor..chlorenchyma | mr.....medullary ray |
| f.....pericyclic fibres. | px.....protoxylem |
| ph.....phloem | p.....pith. |
| pf.....phloem fibres. | |

The section shows a single bundle from a stem of an age suitable for propagating: tissues fully differentiated and lignified, but no secondary changes.

Figure 20.

LONGITUDINAL SECTION OF THE APEX OF CLEMATIS UNGUICATA.

Epidermis and sunken stoma of Clematis afoliata. The cuticle is indicated by a heavy line.

At the immediate apex (a) the starch is distributed uniformly throughout the tissues.

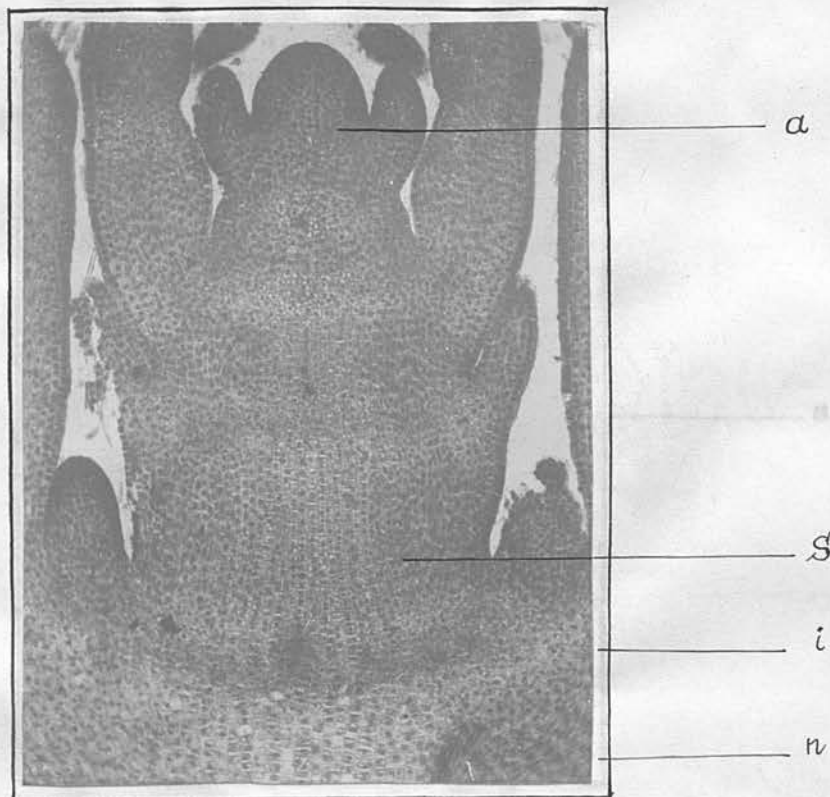
The starch-sheath is differentiated about the level of a, and below this point the starch is, in the young stem, confined to the cortex in the internodes (i), but travels to the pith at the nodes (n).

Figure 21.

LONGITUDINAL SECTION OF THE APEX OF CLEMATIS UNCINATA.
 VASCULAR BUNDLE FROM THE STEM OF

Microtome section, 12μ , stained Bismark Brown.
 Negative on E.C.Ortho film, Wratten "C" filter.

Hand cut section, $\times 28$ Iodine, Gentian Violet, Light green.
 Negative on E.C.Ortho film, Wratten "C" filter. \times



Young stem, the bundles fully differentiated but not yet fully lignified. The starch sheath (s) still prominent.

At the immediate apex (a) the starch is distributed uniformly throughout the tissues.

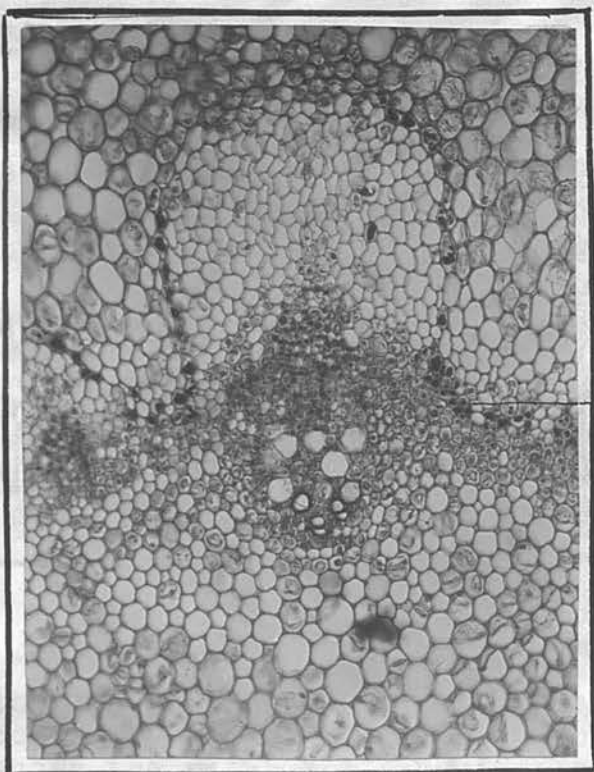
The starch-sheath is differentiated about the level of s, and below this point the starch is, in the young stem, confined to the cortex in the internodes (i), but travels to the pith at the nodes (n).

Figure 22.

VASCULAR BUNDLE FROM THE STEM OF

CLEMATIS HERACLAEOFOLIA.

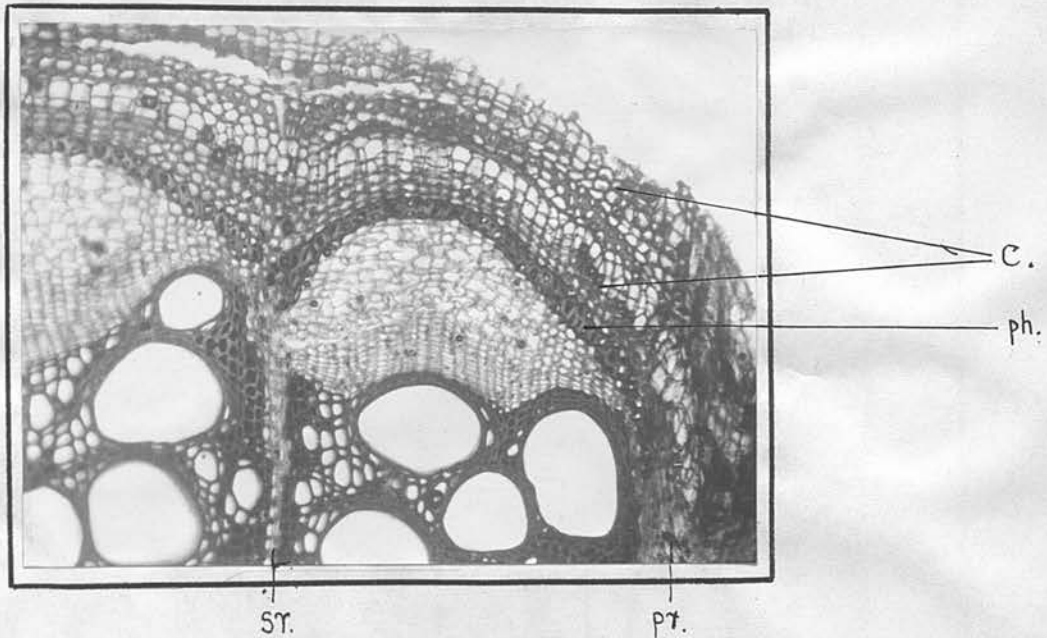
Hand cut section, stained Iodine, Gentian Violet, Light green.
 Negative on E.C.Ortho film, Wratten "G" filter. x



Young stem, the bundle fully differentiated but not yet fully lignified. The starch sheath (s) still prominent.

Figure 23.PERIDERM FORMATION IN AN OLD STEM OF GLEMATIS MONTANA.

Hand section, stained Chlor-zinc-iodine.
Negative on E.C.Ortho film, no screen. x 77.



In the second and subsequent years the phellogen cuts off the corresponding annual group of phloem fibres. This section shows the remains of two phellogens, each of which has produced 3-4 layers of cork cells (c). The phellogen spans both the wide primary rays (pr) and the narrower secondary wood rays (sr). The phloem fibres of the current year are fully differentiated (ph), but the new phellogen has not yet appeared.

Figure 24.SECTIONS THROUGH IMMATURE XYLEM AND PHLOEM OF
GLEMATIS MONTANA.

Sections cut tangentially as near as possible to the cambial zone. Material fixed formalin-alcohol. Sections stained with the 2 sol. Chlor-zinc-iodine. Drawn with Leitz oc.III, objective 6. Reichert camera-lucida. x 400.

A. Immature Xylem.

- a.... a fully differentiated tracheid, lignified wall, bordered pits, no cell contents.
- b.... a partially differentiated tracheid, lignified wall, simple pits, nucleus (n), protoplasm, and starch grains.
- c.... a cell with thickened cellulose walls, incipient pits, nucleus, protoplasm.
- d.... a cell with thin cellulose walls, no pits, nucleus and protoplasm.

B. Immature Phloem.

- e.... young **sieve**-tube, showing perforations of **sieve**-plate (sp).
- f.... phloem parenchyma, derived from the cambial initials by transverse as well as radio-longitudinal divisions.

Both have cellulose walls.

Photograph of the cells drawn in A, (b and c).

Magnif. 270.



Fig. 24.

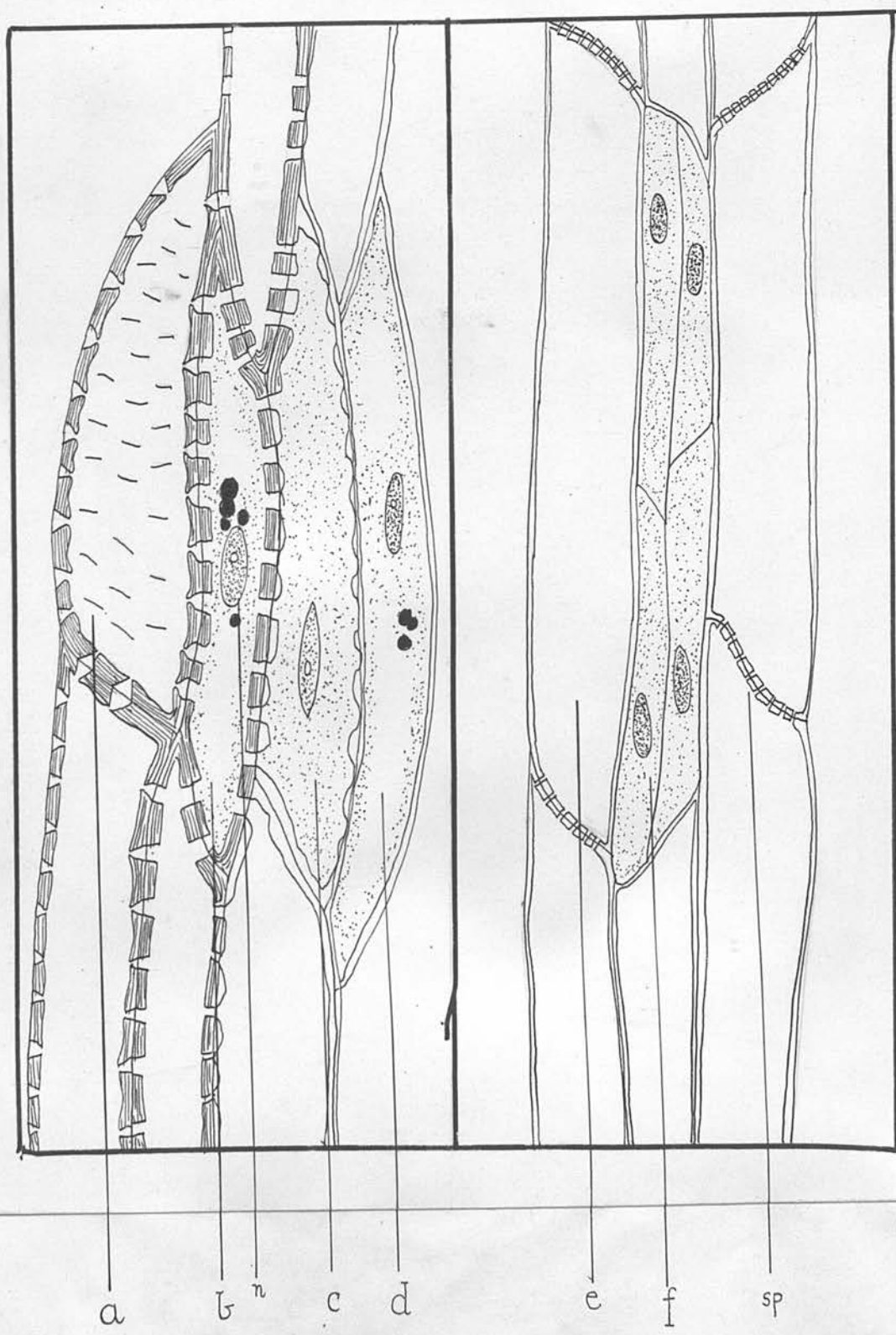


Figure 25.TRANSVERSE SECTION OF A WOODY STEM OF CLEMATIS MONTANA.x

Details of secondary wood, rays and pith. The section includes two wide primary rays flanking a large bundle, which shows two (or possibly three) ill-defined annual rings, with very large pitted vessels.(v). The beginning of the ray proper is marked by the dense accumulation of starch.(s). The shape and pitting of the pith cells is well seen at (p).

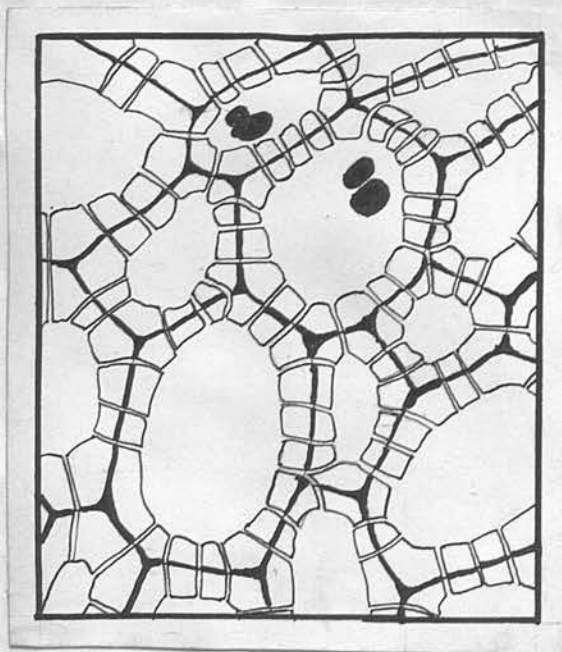
Figure 26.

RADIAL LONGITUDINAL SECTION OF A WIDE RAY OF CLEMATIS MONTANA AT ITS JUNCTION WITH THE PITH. x 77.

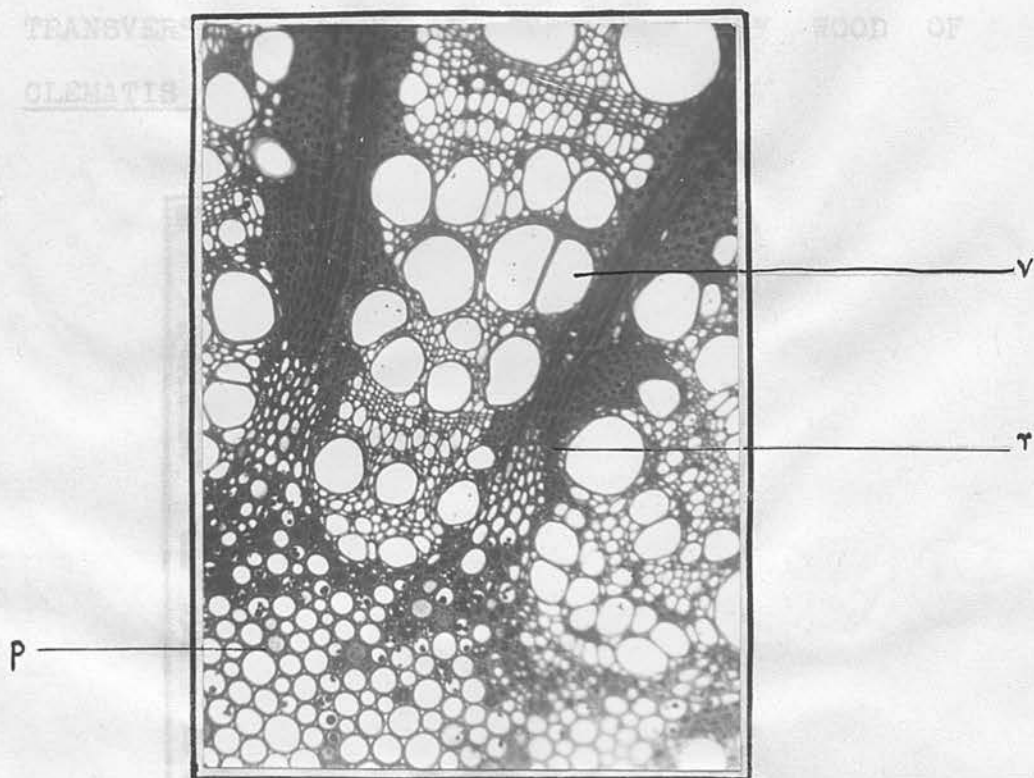
The long axes of the ray cells are horizontal and radial, at right angles to the vertically elongated pith cells. The rays cells are lignified and show numerous simple pits.

Figure 27.

Details of the ray cells on a larger scale.



T.S. WOODY STEM OF CLEMATIS MONTANA.
Hand section, stained Chlor-zinc-iodine. x 77.



R.L.S. WIDE RAY OF CLEMATIS MONTANA, AT ITS JUNCTION WITH
THE PITH. x 77.
Figure 26.

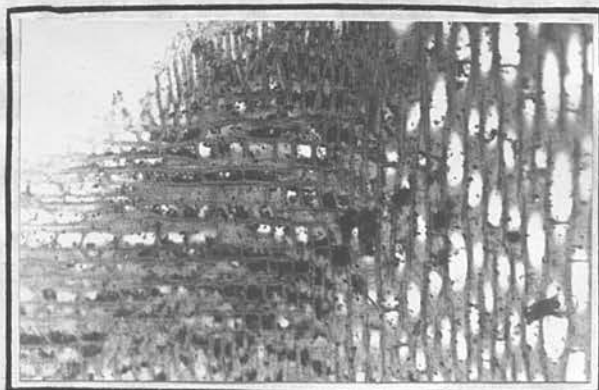
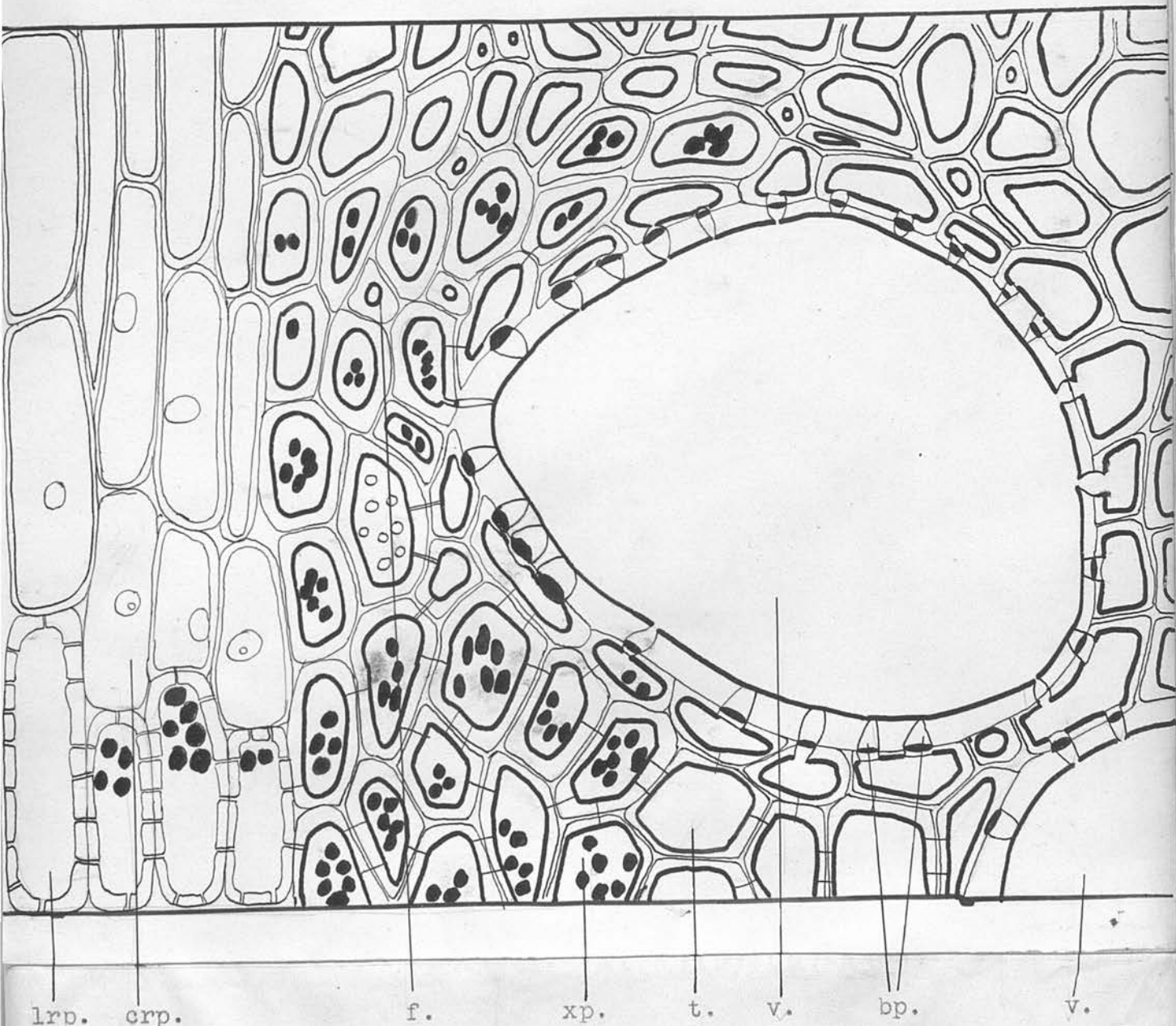


Figure 29.

Details of the secondary wood of Clematis montana.

- v..... large pitted vessel.
- t..... tracheid.
- xp..... xylem parenchyma.
- f..... fibre.
- lrp..... lignified ray parenchyma.
- crp..... ray parenchyma with cellulose walls.
- bp..... bordered pit.

Figure 30.

RADIAL LONGITUDINAL SECTION THROUGH THE WOOD OF
CLEMATIS SMILACIFOLIA.

x 77.

Hand cut section, mounted in phloroglucin and HCl.
 Negative on E.C.Ortho film, Wratten "B" filter.

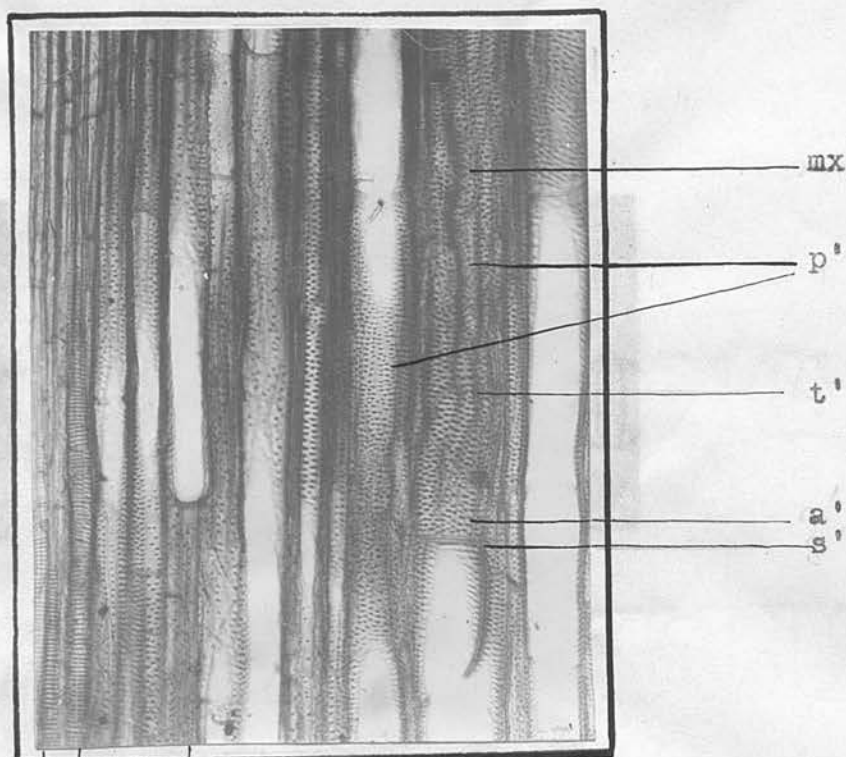
Shows the very large (150μ diam). pitted vessels
 characteristic of the metaxylem of the genus.

- px..... the annular and spiral vessels of the protoxyl
 mx..... a large vessel showing bordered pits (p');
 (the aperture of the pit is oval, and the long
 axis is often at an angle with the aperture
 of the adjacent pit, giving a crossed effect
 at certain foci (a'));
 the remains of a transverse septum (s') per-
 sisting as a collar:
 wavy lines of tertiary thickening (t').
 par..... xylem parenchyma, with narrow lumen, simple
 pits, and obtusely pointed ends. The normally
 abundant starch has been cleared with potash.

Figure 30.

RADIAL LONGITUDINAL SECTION THROUGH THE WOOD OF
CLEMATIS SMILACIFOLIA.

x 77.



- Name.
1. C. smilacifolia 23.10.23. 20.11.23. Rooting from stem above and out. Very little callus.
 2. C. sp. 23.10.23. 20.11.23. Root coming from callus.
 3. C. Halerii 23.10.23. 20.11.23. Beginning of callus formation.

Figure 32.

Figure 31.

TRANSVERSE SECTION OF A CUTTING OF CLEMATIS SMILACIFOLIA.

CUTTINGS OF CLEMATIS TO SHOW EARLY STAGES OF
CALLUS AND ROOT FORMATION.



1.



2.



3.

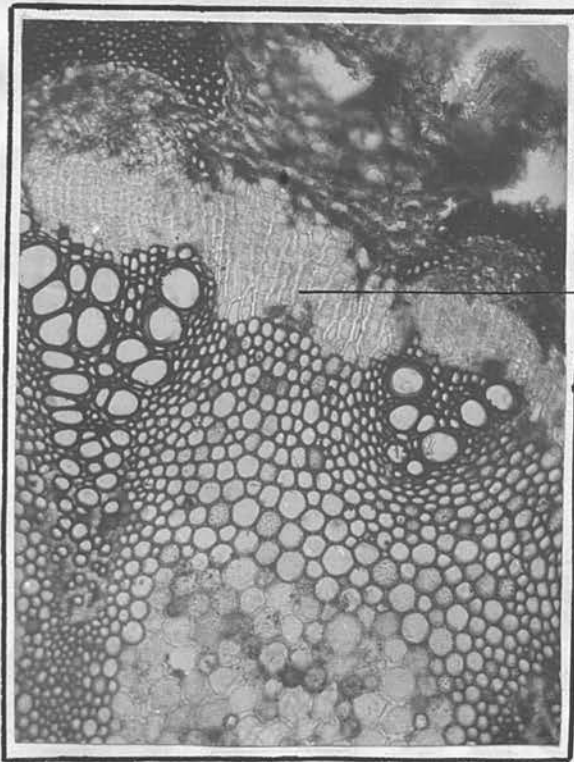
<u>Name.</u>	<u>Inserted.</u>	<u>Photographed.</u>
1. <u>C. smilacifolia.</u>	23.10.23.	20.11.23. Rooting from stem above the cut. Very little callus.
2. <u>C. Spooneri.</u>	23.10.23.	20.11.23. Root coming from callus.
3. <u>C. Hilarii.</u>	23.10.23.	20.11.23. Beginning of callus formation.

the beginnings of callus activity in the interfascicular
cambium. (a).

Figure 32.

TRANSVERSE SECTION OF A CUTTING OF CLEMATIS SMILACIFOLIA,
SHOWING A VERY EARLY STAGE IN CALLUS FORMATION.

x 77.



C.

Hand section, stained Safranin and Gentian Violet.

The section was cut after the cutting had been in the propagating frame at 65° F. for 13 days. It shows the beginnings of callus activity in the interfascicular cambium. (c).

Figure 33.

RADIAL LONGITUDINAL SECTION OF A CUTTING OF
CLEMATIS SMILACIFOLIA SHOWING A VERY EARLY STAGE
 IN CALLUS FORMATION. x 700,

Hand section, stained Safranin, Gentian Violet, Orange G. Drawn with Leitz ocular 3, objective 6, tube-length 170 mm., using a Reichert camera lucida, the drawing made at the level of the table.

The cutting was kept in a frame at 65°F. for eight days. The material was cut fresh, and the sections fixed in Blés fluid.

Shows the first signs of abnormal divisions in the interfascicular cambium (compare with previous figure.)

- d.... dead cells through which the knife has passed. Remains of protoplasm as brownish traces. Injury extends above cut surface.
- i.... injured cells with contracted protoplasm.
- p.... partially differentiated cells at the periphery of pith.
- c,c.. cambium layer and its immediate derivatives.
- w.... oblique tangential wall.
- m.... cells of medullary ray.

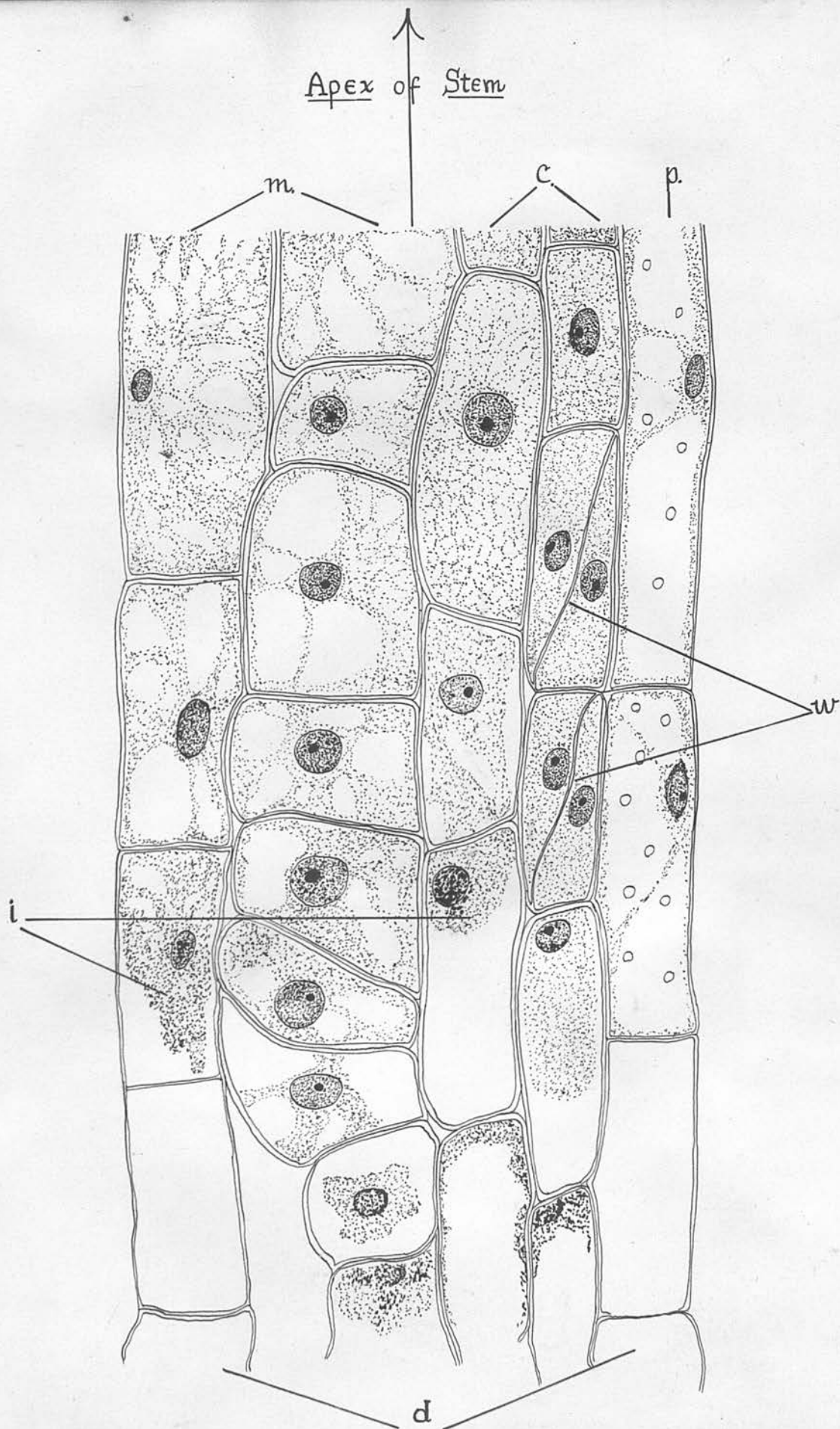
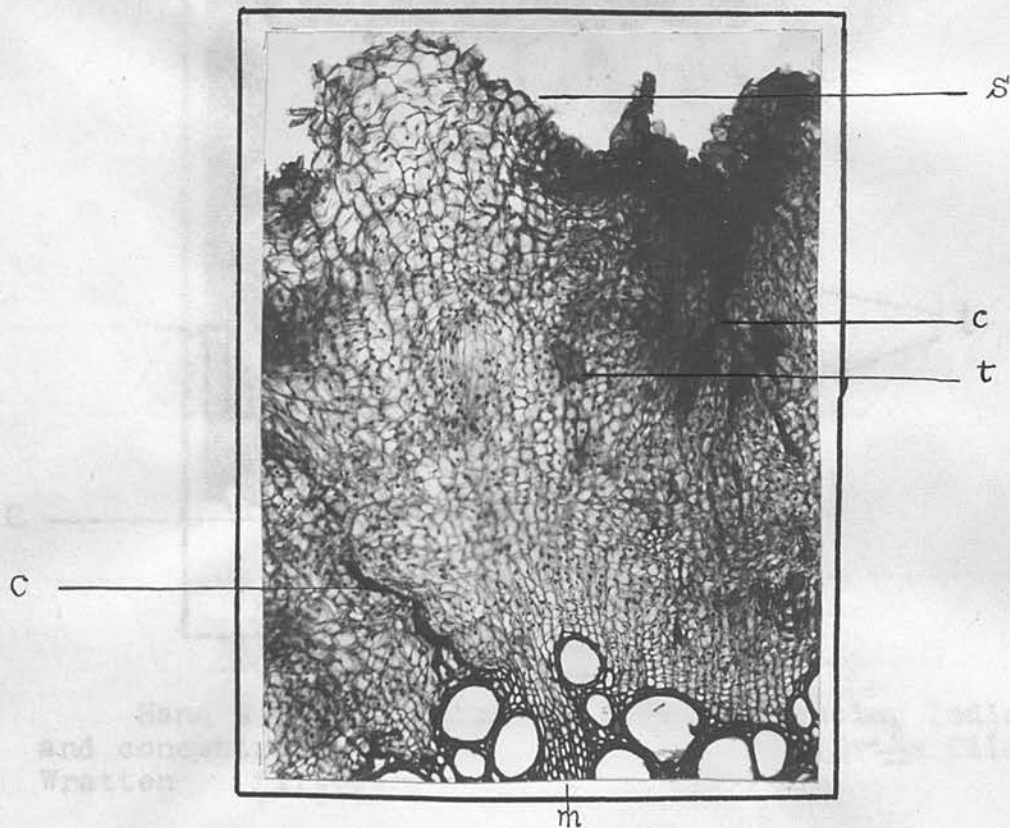


Figure 34.

TRANSVERSE SECTION OF CLEMATIS NAPAULENSIS
SHOWING CALLUS FORMATION.

x 54.

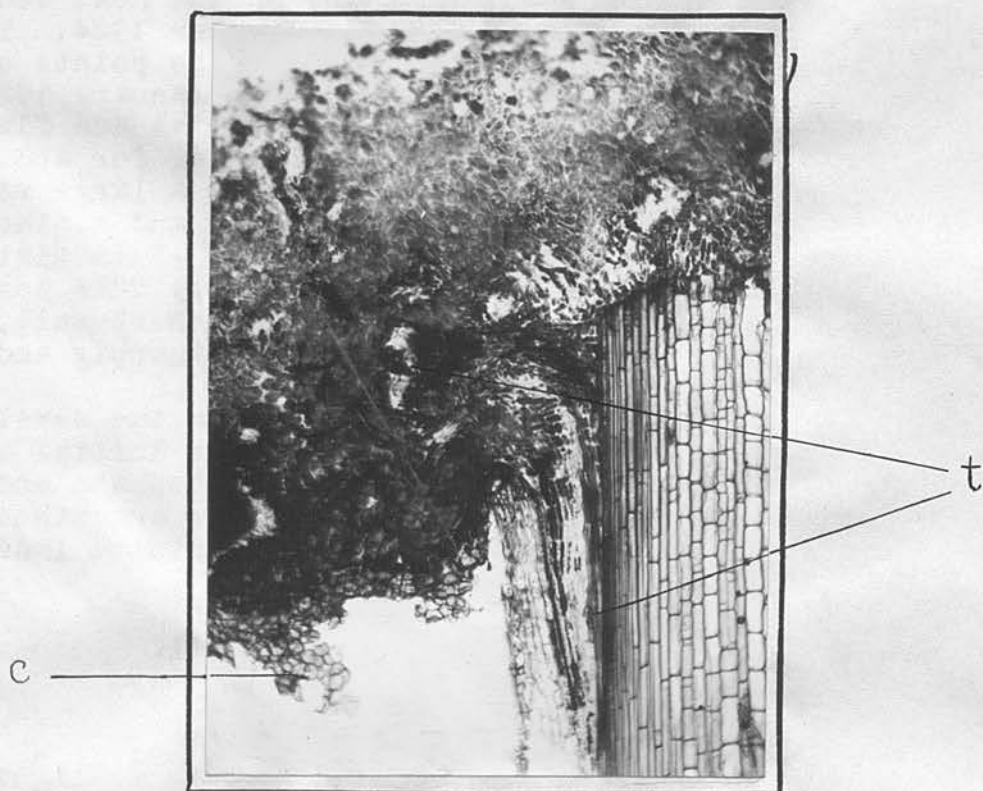
Hand cut section, stained Gentian Violet and Light Green. Negative on E.C.Ortho film, Wratten "G" filter.



The callus originates in the medullary rays and grows outward in lobed masses: at c,c, are crushed cells where two lobes have grown together. The fascicular cambium then becomes involved, and the whole of the phloem, pericyclic fibres and cortex are sloughed off. The callus in this section is still active and has not become corky. At s, are seen the characteristic rounded cells of the free surface. Note the prominent nuclei of the callus cells, and the nests of tracheids(t) developing in the new growth.

Figure 35.

LONGITUDINAL SECTION OF A STEM CUTTING OF CLEMATIS GRACIFOLIA
SHOWING A WELL DEVELOPED CALLUS. x 28.



Hand section, treated with Phloroglucin, Iodine and concentrated H_2SO_4 . Negative on E.C.Ortho film, Wratten filter.

With this mixture of reagents, the callus gives an intense cellulose reaction, except where it has become corky at the outside (c). This section shows the part played by the cambium in building up the callus. The pith in this case is entirely lignified, and does not contribute to the callus. There is a development of secondary tracheids by the cambium where it passes over into callus, and tracheids are also differentiated irregularly in the callus itself. (t).

Figure 36.

T.S. STEM OF CLEMATIS SMILACIFOLIA TO SHOW THE ORIGIN
OF ADVENTITIOUS ROOTS FROM THE VASCULAR CAMBIUM.

A. The cutting from which this and the next section were made was inserted on 24th. November 1924. The root initials were just visible as white points on the green surface of the stem on 5th. January 1925. The sections were cut from fresh material and fixed after cutting in formalin-acetic-alcohol for about three hours. They were then placed in a large volume of 90% alcohol to wash over night, and stained the next morning with Gentian violet (1% in distilled water) and Light Green (in Clove Oil). This combination differentiates the vascular structure well, and at the same time picks out the nuclei sharply and stains starch lightly.

The figure shows an early stage in the development of an adventitious root. The root initial is unmistakable by reason of its dense cytoplasm and large nuclei. Even at this stage there are signs of organization in the meristem and definite indications of polarity.

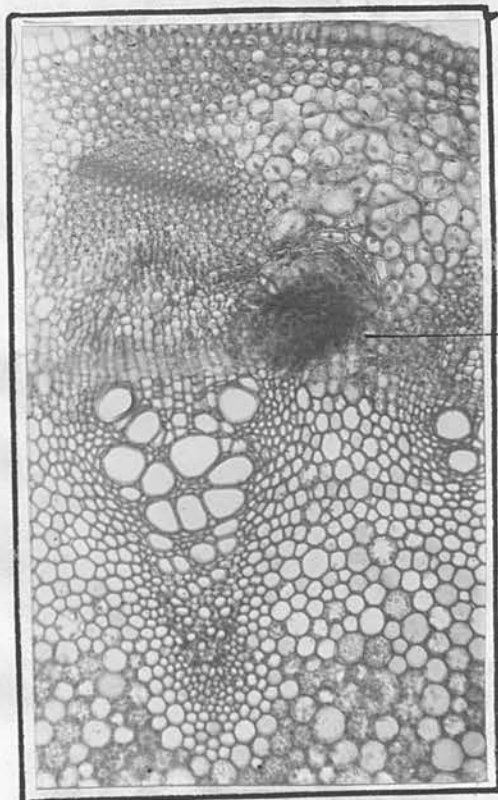
r_1 root initial.

B. This figure shows a root initial at a slightly later stage, when the root is beginning to orient itself and to grow along a radius of the stem. In all the cases examined, the emergent roots behave in this way, instead of continuing their growth in a line with the cambial arc, as it begins.

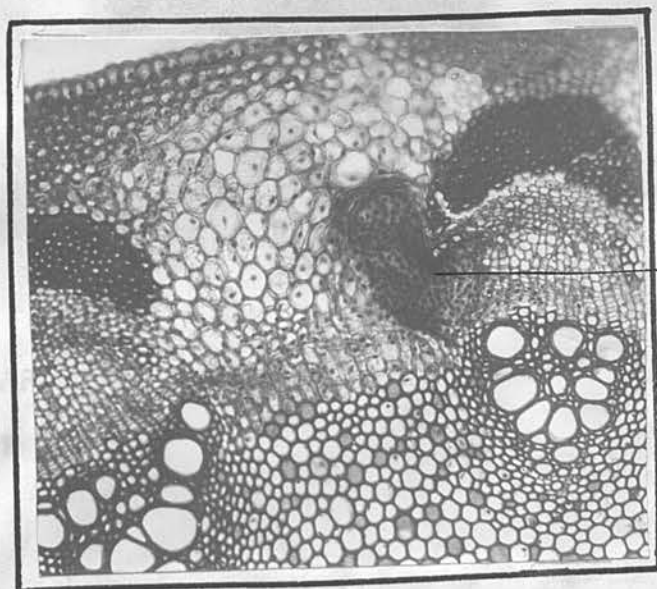
r_2 older root initial.

Both negatives on E.C.Ortho film, A with no light filter, B with Wratten "B&G" filters.

x 77.

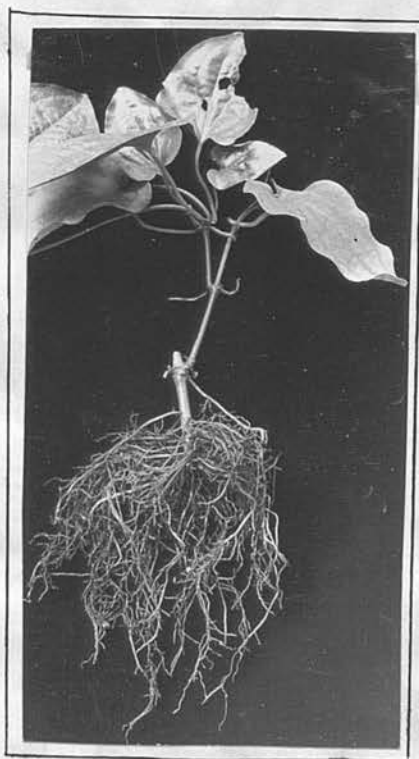


A.



B.

Figure 37.



ONE YEAR OLD CUTTING OF CLEMATIS SMILACIFOLIA
TO SHOW THE ROOT DEVELOPMENT.

Figure 38.

VASCULAR CONNECTION TO THE ADVENTITIOUS ROOT.

Hand section, stained Gentian Violet and Light Green.
Negative on E.C.Ortho Film, no screen. x 77.

The cutting from which this section was made is shown
in the previous figure.

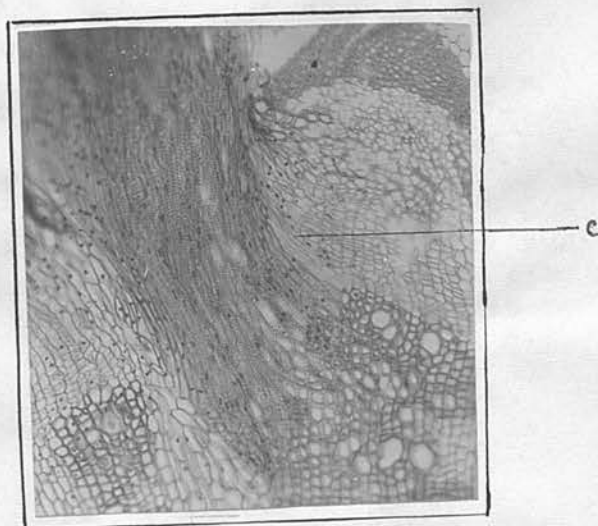
A. The section shows the mass of tracheids which connect the vascular supplies of the root and the stem, and their connection with one of the bundles of the stem. The structure of the original bundle still shows plainly.

The cambiums of the stem and the root are continuous.
(c). Note the numerous transitional elements in the xylem of the root, which have lignified walls and yet show nuclei. The second year's growth of wood in the stem bundle is irregular, and shows fewer large vessels than the first year's growth.

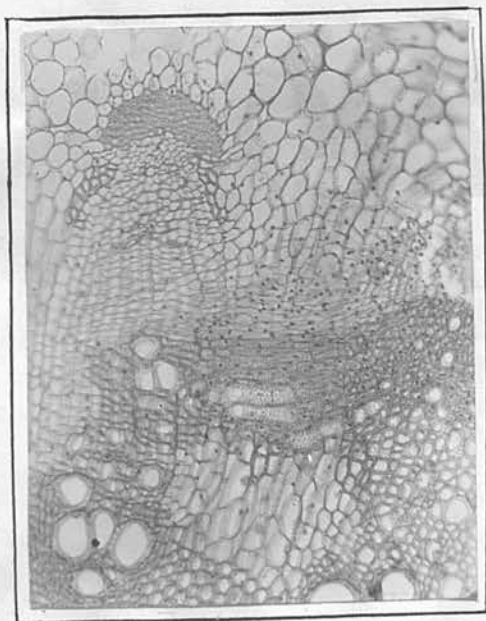
Owing to the downwardly directed growth of the root, it is not possible to get a section transverse to the stem and at the same time median to the root.

B. Cross-grained wood produced above and below the adventitious root.

Figure 38.



A.



B.

Figure 39.BLANCHED CUTTINGS OF CLEMATIS SMILACIFOLIA.

The stems from which the cuttings were made were etiolated for eighteen days. The cuttings were put in the frame on 17.11.23. One was made (B) at the internode in the regular way, while the other (A) was made 1 cm. below the node. On 30.11.23. both cuttings were well callused over, and A was cut hard back to the leaf-bases. This accounts for the greater development of roots on B. The photograph was taken on 3.1.24.

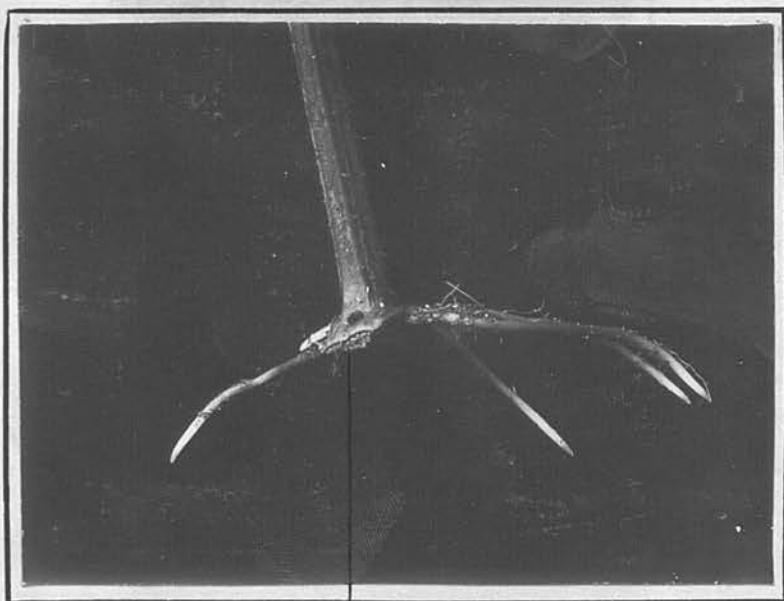


BLANCHED CUTTINGS OF CLEMATIS SMILACIFOLIA.

Figure 39. Blanching cuttings of Clematis smilacifolia, showing the roots (a), and roots (b) freely through the leaf-tissue.

Figure 40.

BLANCHED NODAL CUTTING OF CLEMATIS SMILACIFOLIA.



Basal end of a blanching nodal cutting of Clematis smilacifolia, to show callus (c), and roots coming freely through the leaf-bases.

Figure 41.

BLANCHED NODAL CUTTING OF CLEMATIS SMILACIFOLIA:
later stage.



The stem from which the cutting was made was painted with Indian ink on 20.10.23. Cutting made 7.11.23. By 1.2.24. the cutting was well rooted and potted. The ink still adhered to the stem, so that growth at first was exceedingly slow, and the first leaf to expand was etiolated and deformed.(1). Subsequent growth and development was however normal. Photographed 19.10.24.

At a,.... are seen cells which no longer stain.

b,.... collared cells which still stain red.

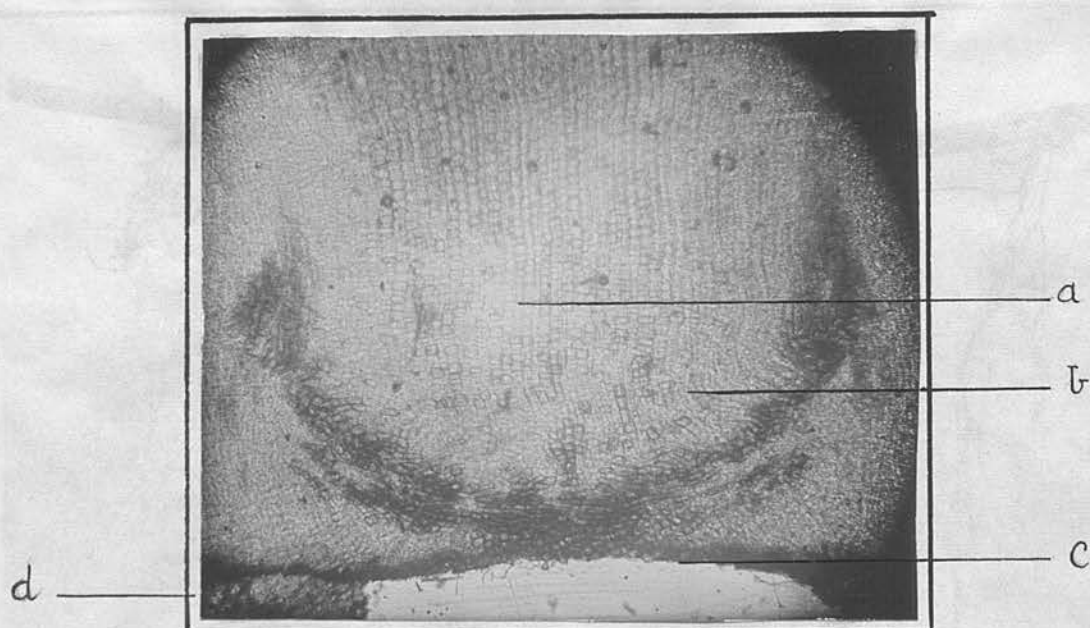
c,.... the cell surface.

d,.... beginning of callus, the cells of which have partly grown over the cut end.

Figure.42.

MEDIAN LONGITUDINAL SECTION OF AN ETIOLATED
 NODE OF CLEMATIS SMILACIFOLIA.

OLD Hand cut section, mounted in phloroglucin+HCl.



The cutting from which this section was made is shown at A in Figure 39.

After etiolation the cells of the pith and the fibres stain much less intensely (or not at all) with phloroglucin and HCl.

At a,.... are seen cells which no longer stain.

b..... scattered cells which still stain red.

c,.... the cut surface.

d,.... beginnings of callus, the cells of which have partly grown over the cut end.

Figure 43.

NATURAL CASE OF ROOTING AT THE NODE IN AN
OLD STEM OF CLEMATIS MONTANA.

